Review

Sources of Extracellular Tau and its Signaling

Jesús Avila^{a,b,*}, Diana Simón^{a,b}, Miguel Díaz-Hernández^c, Jesús Pintor^d and Félix Hernández^{a,b}

^a*Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Madrid, Spain*

^b*Centro de Investigación Biomédica en Red de Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain*

^c*Department of Biochemistry and Molecular Biology IV, Fac. Veterinary, Complutense University of Madrid, Madrid, Spain*

^d*Department of Optics II (Optometry and Vision), Faculty of Optics and Optometry, Complutense University of Madrid, Madrid, Spain*

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Abstract. The pathology associated with tau protein, tauopathy, has been recently analyzed in different disorders, leading to the suggestion that intracellular and extracellular tau may itself be the principal agent in the transmission and spreading of tauopathies. Tau pathology is based on an increase in the amount of tau, an increase in phosphorylated tau, and/or an increase in aggregated tau. Indeed, phosphorylated tau protein is the main component of tau aggregates, such as the neurofibrillary tangles present in the brain of Alzheimer's disease patients. It has been suggested that intracellular tau could be toxic to neurons in its phosphorylated and/or aggregated form. However, extracellular tau could also damage neurons and since neuronal death is widespread in Alzheimer's disease, mainly among cholinergic neurons, these cells may represent a possible source of extracellular tau. However, other sources of extracellular tau have been proposed that are independent of cell death. In addition, several ways have been proposed for cells to interact with, transmit, and spread extracellular tau, and to transduce signals mediated by this tau. In this work, we will discuss the role of extracellular tau in the spreading of the tau pathology.

Keywords: Exomas, muscarinic receptors, phosphotau, tau secretion, tau spreading

INTRODUCTION

Tau is a brain microtubule associated protein that when bound to microtubules suppresses microtubule dynamics (assembly-disassembly) [1]. The presence of stabilized microtubules could facilitate the formation of neurite-like cytoplasmic extensions [2]. However, tau may be modified by phosphorylation and this modification of tau can compromise its capacity to bind microtubules [3].

Alzheimer's disease (AD) is characterized by the formation of senile plaques, neurofibrillary tangles, and the death of (mainly cholinergic) neurons [4, 5].

Neurofibrillary tangles are aggregates of paired helical filaments and the main component of these filaments is the tau protein [5]. The distribution of plaques and tangles as AD progresses had been analyzed in several studies, demonstrating a correlation between the progression of the disease and that of the tau pathology [6]. Also, it was recently suggested that some specific forms of the amyloid peptide could be responsible for the spreading of the amyloid pathology [7] and that extracellular tau protein could be responsible for the spreading of the tau pathology [5, 8, 9].

In AD, large scale death of cholinergic neurons (these bearing nicotine or muscarinic receptors) occurs [4], which might produce the release of intracellular tau to the extracellular milieu, which in turn could be toxic to other cells. Indeed, extracellular tau has been found in the cerebrospinal fluid (CSF) of AD patients, which

*Correspondence to: Jesús Avila, Centro de Biología Molecular Severo Ochoa (CSIC-UAM), 28049 Madrid, Spain. Tel.: +34911964564; Fax: +34911964715; E-mail: javila@cbm.uam.es.

may be the consequence of cell death [10]. However, in a mouse model, the presence of tau in the CSF was proposed to be due to an active process of secretion *in vivo* [11, 12]. Thus, extracellular tau could accumulate not only by cell death but also in other ways [9, 13]. Here we present a brief review of the possible mechanism by which extracellular tau can spread.

INTRACELLULAR TAU PROTEIN AUGMENTS IN ALZHEIMER'S DISEASE

It was more than twenty years ago that an increase in the amount of intracellular tau was described in AD patients as opposed to non-demented controls [14]. This increase in tau could be toxic to neurons and indeed, a reduction in the amount of intracellular tau has a protective effect in mouse models of neurodegeneration [15–17]. Nevertheless, we still know little about the factors that provoke an increase in tau expression, although such effects may be provoked by FGF and Dyrk1A [18, 19].

REGULATION OF THE AMOUNTS OF INTRACELLULAR TAU

As described previously, an increase in the amount of intracellular tau could be toxic to neurons [15–17]. However, it has also been proposed recently that an intercellular “glymphatic” pathway [20] could be responsible for clearing the excess extracellular tau that could arise from axon damage [21]. It was suggested that CSF is exchanged with interstitial fluid (ISF) in the brain through a pathway involving a para-arterial CSF influx route and a para-venous ISF route [20, 22]. In this way, tau protein may be found in the CSF in the absence of cell death.

An increase in the amount of intracellular tau could reflect an increase in the synthesis of the protein or a decrease in its degradation. An increase in the amount of tau messenger RNA in the brain of AD patients was described long ago [23].

Two main mechanisms contribute to the degradation of the tau protein, the ubiquitin-proteasome pathway and the autophagic-lysosomal pathway [24–27]. Indeed, the proteasome may degrade natively unfolded tau as it does for other proteins. In addition, it has been suggested that tau may be rapidly ubiquitinated under conditions of stress, whereas such ubiquitination occurs much more slowly under normal conditions [28]. It was recently shown that a higher concentration of intracellular tau can interfere with the quality

control exercised on this protein in the endoplasmic reticulum that may increase the ubiquitinated proteins found in the brain, which may be toxic to the cell [8]. Alternatively, tau clearance through lysosomal processing has also been reported [29]. It is also known that autophagy may occur by chaperone-mediated autophagy, whereby the proteins to be degraded generally contain a pentapeptide motif that facilitates their translocation to lysosomes through the Hsc70 protein. Tau contains two such peptide motifs: residues 336–340 and 347–351 [28].

In addition to the two aforementioned mechanisms to remove excess tau protein, a third mechanism to clear tau also exists [30], involving its secretion from the cell once a certain threshold of intracellular tau has accumulated. Tau may be secreted in its free form or in coated membrane vesicles [30], and the former is to some extent dependent on intracellular calcium as it is impaired in the presence of 2 μ M AM-BAPTA. This secreted tau may persist in the extracellular space.

THE SPREADING OF THE TAU PATHOLOGY

It was suggested in 2006 that extracellular tau would be only present in neurodegenerative disorders and not in healthy individuals, and that it is the toxic agent involved in the spreading of such pathologies [31]. However, different ways by which the toxic extracellular tau appears have since been described. As indicated previously, one event that might explain the presence of extracellular tau is cell death. Similarly, tau secretion provoked by an increase in the intracellular concentration of tau, or due to tau aggregation, is another way it may accumulate extracellularly that does not involve neuron death.

In AD, intracellular tau may be modified by phosphorylation and it could aggregate into aberrant polymers (for a review, see [3]). The phosphorylated intracellular tau could be toxic to the neuron [32, 33] and it is also discussed if aggregated tau could damage the cell [34].

The role of extracellular tau in AD is less clear and depending on the coincidence with cell death, the characteristics of the extracellular tau may be distinct. Secreted tau could be released “naked” or “clothed” in membrane vesicles that is secreted as the free cytosolic protein or bound to cell membranes [35, 36]. The latter will be secreted in exocytotic vesicles whereas, upon cell death, intracellular tau, whether in its modified or aggregated form, will be released naked into the extra-

cellular space. In addition, tau coated by membrane vesicles will interact in a different way with surrounding cells than uncoated tau, which could interact with a cell through specific cell receptors. It has also been proposed that extracellular tau aggregates could enter a cell through active endocytosis [9, 37, 38]. Alternatively, it is unclear whether the cells that can bind tau are of only a single cell type (e.g., neurons), or if tau can bind non-specifically to all cells. In the first case, tau may be required to bind to cells through specific receptors. Thus, the presence of such receptors will define the specificity of tau binding to a cell. There is data suggesting that both possibilities may occur and that the release of tau to the extracellular space may or may not be dependent on cell death [9, 13, 31, 39, 40, 41–43]. Indeed, it is also unclear if soluble tau (monomeric or present in small aggregates) is a toxic agent [31, 41], or if larger tau aggregates (or tau with a specific conformation) are the truly toxic conformations [9, 13, 39, 40].

TAU MONOMERS VERSUS TAU AGGREGATES

As indicated above, it remains unclear whether tau toxicity is due to the presence of monomers, oligomers or large aggregates, such as paired helical filaments or neurofibrillary tangles. There are several indications that neurodegeneration occurs before the formation of tangles in AD or in models of this disease [31, 44–48]. Indeed, it has been proposed that tangles could be protective and their presence could delay cell death [31, 34, 44–48]. Moreover, when the effect of soluble or aggregated tau (in paired helical filaments) was tested, greater toxicity was attributed to soluble tau than to PHF-tau [31]. However, when the soluble tau fraction was analyzed, both tau monomers and dimers or trimers were identified, and it is not clear which might be the main toxic agent. In fact, there is even some evidence suggesting that small tau oligomers could be those involved in cell toxicity [38, 49–52].

PHOSPHOTAU VERSUS UNMODIFIED TAU

Although, it was suggested that modified forms of extracellular tau could be toxic, it is now clear that unmodified extracellular tau [53] and synthetic unmodified tau are toxic agents *in vivo* [39]. Indeed, in AD intracellular tau might exist mainly in a phosphorylated form and the phosphorylation of tau could increase its secretion into the external space [54]. However, extra-

cellular tau is largely thought to be dephosphorylated [55], which could be due to the presence of extracellular phosphatases like the tissue non-specific alkaline phosphatase (TNAP) [53]. Significantly, an increase in TNAP in both brain tissue and plasma was recently described in AD patients [56], consistent with previous data [54]. In addition, unmodified tau can bind to muscarinic M1/M3 cell receptors, thereby promoting cell damage, while phosphorylated tau does not bind to these receptors [41].

TAU TRANSMISSION

Several models for the transmission of tau from one cell to another cell have been proposed. In one of these models, tau transmission requires cell death and the subsequent interaction of the tau released with a neighboring cell [31]. By contrast, another model proposes the release of tau when intracellular tau is overexpressed, even in the absence of cell death, and that this secreted tau possibly interacts with surrounding cells [30]. More recently, it was suggested that the release of endogenous tau in the absence of cell death is stimulated by neuronal activity [55]. Significantly, the release of fibrillar tau to the extracellular space and the uptake of that aggregated tau via macropinocytosis by recipient cells has been described, a process in which heparin sulfate proteoglycans may be implicated [57]. Finally, there is some evidence that tau may be transmitted via synapses [58, 59].

DYNAMIN VS CLATHRIN

In terms of the propagation of the tau pathology, it still remains unclear whether extracellular tau can be internalized by endocytosis. It has been proposed that soluble, uncoated (“naked”) tau could be internalized by cells via receptor-mediated endocytosis [42]. However, tau can also be internalized independently of such events, since inhibition of clathrin-mediated endocytosis does not block tau uptake under certain conditions [37]. Thus, the uptake of tau could be driven by dynamin [37]. Also, pinocytosis has been suggested as a means to internalize tau aggregates [9].

EXTRACELLULAR TAU SIGNALING

As indicated previously, a specific interaction between extracellular soluble tau and cell receptors has been proposed through the muscarinic M1/M3 receptors [31, 41]. This result has been also shown in a rabbit

model *in vivo* (Martínez-Aguila A et al, J Alzheimers Dis. 2014, in press). The consequence of tau binding to muscarinic receptors adding purified human tau to the eyes of a rabbit, is to promote tear secretion.

Tau addition may result in an increase in intracellular calcium, which in turn may facilitate protein secretion. If such an increase in calcium is excessively high it may even provoke cell death and the consequent release of

Fig. 2. Extracellular tau peptide facilitates endogenous tau release. A) Scheme of the addition of the tau peptide to HEK-293 cells expressing tau and the M1 muscarinic receptor, and the consequences on tau secretion. B) Protocol to isolate extracellular vesicles. Synthetic human tau peptide containing residues 390–423 was added to HEK-293 cells in culture expressing tau protein [30] and the M1 muscarinic receptor. The cells were then incubated for an additional 24 h and the cell medium was collected. C) Equal amounts of medium, determined by the serum albumin (SA) content, were boiled and the a) heat-resistant fraction of the medium was resolved by electrophoresis (total tau). In addition, the cell medium was centrifuged and the supernatant and the pelleted fractions were isolated. Panel b) shows the electrophoresis of the boiled supernatant of the centrifuged medium to remove the membrane (or vesicle-containing) fraction [30] and c) the pelleted vesicle fraction. Flotilin (F) was used as a protein loading marker for the membrane fraction. HEK-293 cells expressing the M1 receptor (+) or not (–), and in the presence (+) or absence (–) of the tau peptide 390–423, were tested for tau secretion.

intracellular tau to the external space where it could be toxic [42]. If repeated, this process could provide a way to spread the tau pathology.

THE INTERACTION OF A SYNTHETIC TAU PEPTIDE WITH CELL RECEPTORS INDUCES TAU SECRETION

The region of tau involved in its binding to muscarinic receptors has been mapped to a peptide

containing residues 390 to 423 of the largest human tau isoform in the central nervous system [41] (Fig. 1). To test if the addition of extracellular tau may promote the release of intracellular tau, a synthetic tau peptide containing residues 390 to 423 was added to a culture of non-neuronal HEK-293 cells expressing the tau protein [30] and the M1 muscarinic receptor. The consequence was that the intracellular human tau present in the neuroblastoma cells was released into the extracellular medium (Fig. 2), in the absence of significant

Fig. 3. Extracellular tau and AβPP proteolysis. A) Human neuroblastoma cells (SH-SY5Y) were incubated with or without carbachol in the presence or absence of atropine, and AβPP cleavage was assessed in western blots probed with the AβPPct antibody. B) Human neuroblastoma cells were exposed to the tau peptide (390–423aa) for 24 h and the AβPP (and its proteolytic peptides) accumulated in the cells was assessed by probing western blots with the 4G8 antibody. A slight increase in some AβPP peptides (see arrows) was found. C) The epitopes in the AβPP molecule recognized by the 4G8 and AβPPct antibodies are shown.

Fig. 4. Tau spreading model. Dephosphorylated tau or a tau peptide containing residues 390–423 interacts with M1/M3 muscarinic receptors present on a neuronal cell. This produces an increase in intracellular calcium, which may provoke neurodegeneration if excessive. When the increase in intracellular calcium is lower and it does not provoke cell death, endogenous tau may be secreted. Endogenous tau released to the extracellular medium should be dephosphorylated (probably by TNAP), thereby acting as an agent toxic to neighboring cells: for steps 1 and 2 see references 30 and 40; for step 3, see reference 41; and for step 4 see reference 43.

Fig. 5. Tau transmission. Different pathways of extracellular tau transmission that can be explained by the working hypothesis proposed. Accordingly, in non-pathological situations and/or at early stages of the disease, soluble tau can be transferred through neurotransmitter receptors. At intermediate disease stages, after the appearance of tau filaments, its transmission could take place as tau aggregates in naked or vesicle-coated forms. Finally, at late stages of the pathology, where cell death is clearly evident, extracellular tau could be released from degenerated neurons and it may interact with the surrounding neurons.

cell death. Moreover, no secretion was detected when the tau peptide was added to HEK-293 cells expressing tau but lacking the M1 receptor. Hence, the spreading of tau from cell to cell may not only be dependent on cell death but also, the tau pathology may progress through a mechanism involving the binding of tau to muscarinic receptors in the absence of cell death and the secretion of intracellular tau. Different tau isoforms were seen to be secreted, both in a “naked” form or covered by membrane vesicles, the latter displaying a higher electrophoretic mobility. This phenomenon may reflect the inclusion of tau 3R but not tau 4R in microvesicles [54, 60], or it may be due to distinct level of tau phosphorylation in each of the two secreted fractions [54, 61].

The tau pathology was seen to progress *in vivo* in a transgenic mouse expressing human tau [13]. However, the progression of tau pathology was prevented using an active immunization approach, injecting animals with a tau peptide comprising residues 395 to 406 (two of these residues in a modified form) [62]. This is consistent with a possible role of this tau region in the spreading of the tau pathology. M1/M3 muscarinic receptors are coupled to a G_q protein and this interaction will result in an increase in phospholipase C activity that facilitates the release of inositol 1,4,5-Triphosphate, the mobilization of intracellular calcium and the generation of diacylglycerol [63]. The increase in intracellular calcium and the presence of diacylglyc-

erol may activate protein kinase C [64], which in turn might promote alpha-secretase mediated processing of the amyloid- β protein precursor (A β PP) [65, 66] and the modulation of β -secretase activity [67]. Indeed, it has also been described that cholinergic stimulation of the M1 receptor can activate α secretase [68–70].

EXTRACELLULAR TAU SIGNALING THROUGH MUSCARINIC RECEPTORS AND A β PP CLEAVAGE

Some time ago it was described that the activation of muscarinic receptors M1/M3 facilitates the cleavage of A β PP by α -secretase [68], and we confirmed this in SH-SY5Y neuroblastoma cells using an A β PP antibody (A β PPct) that reacts with the C-terminal region of the molecule (Fig. 3). However, this antibody could cross react with the tau protein and thus, to test if a similar effect was found in the presence of tau, the cleavage of A β PP was analyzed in neuroblastoma cells treated with extracellular tau and using the 4G8 antibody against A β PP. The preliminary results indicate that upon addition of extracellular tau, a minor increase (probably not significant) in A β PP fragmentation occurs (Fig. 2B: Simon D et al., unpublished results). It suggests a different effect in the activation of muscarinic receptors M1/M3 by an agonist like corbachol than for tau protein, as previously suggested [42].

CONCLUSIONS

The binding of tau to muscarinic receptors could produce several toxic effects on a cell, including the secretion of tau protein into the extracellular milieu, which might also be provoked upon neuron death. In both cases extracellular tau will accumulate and it may be found in the CSF, such as in disorders like AD [43]. When the nature of tau in the CSF of these patients was assessed, a high proportion of CSF tau was found to be coated by membranous vesicles at early stages of the disease, whereas a higher proportion of uncoated tau was observed at later stages of the disease [43]. This phenomenon may be correlated with the increase in neural death taking place during the development of the disease.

In summary, we would like to stress that: 1) the binding region on tau molecule for muscarinic receptors is located at residues 390–423; 2) binding of extracellular tau to muscarinic receptors results in an increase in intracellular calcium (Fig. 4); 3) an increase in intracellular calcium could facilitate the release of intracellular tau; and 4) other mechanisms of extracellular tau transmission have been described in this work and in trying to explain a function for all of these; we have drawn up a working hypothesis (Fig. 5). In this, it is suggested that at early stages of the disease, soluble tau could be transmitted from cell to cell, and subsequently, as the disease develops into its middle stages, tau aggregates could also play a role, and at later stages, it is likely that cell death could become involved in the release and transmission of tau.

DISCLOSURE STATEMENT

Authors' disclosures available online (<http://www.j-alz.com/disclosures/view.php?id=2028>).

REFERENCES

- [1] Panda D, Samuel JC, Massie M, Feinstein SC, Wilson L (2003) Differential regulation of microtubule dynamics by three- and four-repeat tau: Implications for the onset of neurodegenerative disease. *Proc Natl Acad Sci U S A* **100**, 9548–9553.
- [2] Caceres A, Kosik KS (1990) Inhibition of neurite polarity by tau antisense oligonucleotides in primary cerebellar neurons. *Nature* **343**, 461–463.
- [3] Avila J, Lucas JJ, Perez M, Hernandez F (2004) Role of tau protein in both physiological and pathological conditions. *Physiol Rev* **84**, 361–384.
- [4] Davies P, Maloney AJ (1976) Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* **2**, 1403.
- [5] Selkoe DJ (2011) Alzheimer's disease. *Cold Spring Harb Perspect Biol* **3**, pii: a004457.
- [6] Braak H, Braak E (1991) Neuropathological staging of Alzheimer-related changes. *Acta Neuropathol* **82**, 239–259.
- [7] Nussbaum JM, Schilling S, Cynis H, Silva A, Swanson E, Wangsanut T, Tayler K, Wiltgen B, Hatami A, Ronicke R, Reymann K, Hutter-Paier B, Alexandru A, Jagla W, Graubner S, Glabe CG, Demuth HU, Bloom GS (2012) Prion-like behaviour and tau-dependent cytotoxicity of pyroglutamylation of amyloid-beta. *Nature* **485**, 651–655.
- [8] Abisambra JF, Jinwal UK, Blair LJ, O'Leary JC, 3rd, Li Q, Brady S, Wang L, Guidi CE, Zhang B, Nordhues BA, Cockman M, Suntharalingham A, Li P, Jin Y, Atkins CA, Dickey CA (2013) Tau accumulation activates the unfolded protein response by impairing endoplasmic reticulum-associated degradation. *J Neurosci* **33**, 9498–9507.
- [9] Frost B, Jacks RL, Diamond MI (2009) Propagation of tau misfolding from the outside to the inside of a cell. *J Biol Chem* **284**, 12845–12852.
- [10] Hampel H, Blennow K, Shaw LM, Hoessler YC, Zetterberg H, Trojanowski JQ (2010) Total and phosphorylated tau protein as biological markers of Alzheimer's disease. *Exp Gerontol* **45**, 30–40.
- [11] Yamada K, Cirrito JR, Stewart FR, Jiang H, Finn MB, Holmes BB, Binder LI, Mandelkow EM, Diamond MI, Lee VM, Holtzman DM (2011) *In vivo* microdialysis reveals age-dependent decrease of brain interstitial fluid tau levels in P301S human tau transgenic mice. *J Neurosci* **31**, 13110–13117.
- [12] Barten DM, Cadelina GW, Hoque N, DeCarr LB, Guss VL, Yang L, Sankaranarayanan S, Wes PD, Flynn ME, Meredith JE, Ahljianian MK, Albright CF (2011) Tau transgenic mice as models for cerebrospinal fluid tau biomarkers. *J Alzheimers Dis* **24**(Suppl 2), 127–141.
- [13] Clavaguera F, Bolmont T, Crowther RA, Abramowski D, Frank S, Probst A, Fraser G, Stalder AK, Beibel M, Staufenbiel M, Jucker M, Goedert M, Tolnay M (2009) Transmission and spreading of tauopathy in transgenic mouse brain. *Nat Cell Biol* **11**, 909–913.
- [14] Khatoon S, Grundke-Iqbal I, Iqbal K (1992) Brain levels of microtubule-associated protein tau are elevated in Alzheimer's disease: A radioimmuno-slot-blot assay for nanograms of the protein. *J Neurochem* **59**, 750–753.
- [15] Roberson ED, Scearce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, Gerstein H, Yu GQ, Mucke L (2007) Reducing endogenous tau ameliorates amyloid beta-induced deficits in an Alzheimer's disease mouse model. *Science* **316**, 750–754.
- [16] Rapoport M, Dawson HN, Binder LI, Vitek MP, Ferreira A (2002) Tau is essential to beta-amyloid-induced neurotoxicity. *Proc Natl Acad Sci U S A* **99**, 6364–6369.
- [17] Dawson HN, Cantillana V, Jansen M, Wang H, Vitek MP, Wilcock DM, Lynch JR, Laskowitz DT (2010) Loss of tau elicits axonal degeneration in a mouse model of Alzheimer's disease. *Neuroscience* **169**, 516–531.
- [18] Tatebayashi Y, Iqbal K, Grundke-Iqbal I (1999) Dynamic regulation of expression and phosphorylation of tau by fibroblast growth factor-2 in neural progenitor cells from adult rat hippocampus. *J Neurosci* **19**, 5245–5254.
- [19] Qian W, Jin N, Shi J, Yin X, Jin X, Wang S, Cao M, Iqbal K, Gong CX, Liu F (2013) Dual-specificity tyrosine phosphorylation-regulated kinase 1A (Dyrk1A) enhances tau expression. *J Alzheimers Dis* **37**, 529–538.
- [20] Nedergaard M (2013) Garbage truck of the brain. *Science* **340**, 1529–1530.

- [21] Zemlan FP, Jauch EC, Mulchahey JJ, Gabbita SP, Rosenberg WS, Speciale SG, Zuccarello M (2002) C-tau biomarker of neuronal damage in severe brain injured patients: Association with elevated intracranial pressure and clinical outcome. *Brain Res* **947**, 131-139.
- [22] Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA, Nagelhus EA, Nedergaard M (2012) A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta. *Sci Transl Med* **4**, 147ra111.
- [23] Barton AJ, Harrison PJ, Najlerahim A, Heffernan J, McDonald B, Robinson JR, Davies DC, Harrison WJ, Mitra P, Hardy JA et al. (1990) Increased tau messenger RNA in Alzheimer's disease hippocampus. *Am J Pathol* **137**, 497-502.
- [24] David DC, Layfield R, Serpell L, Narain Y, Goedert M, Spillantini MG (2002) Proteasomal degradation of tau protein. *J Neurochem* **83**, 176-185.
- [25] Dickey CA, Kamal A, Lundgren K, Klosak N, Bailey RM, Dunmore J, Ash P, Shoraka S, Zlatkovic J, Eckman CB, Patterson C, Dickson DW, Nahman NS Jr, Hutton M, Burrows F, Petrucelli L (2007) The high-affinity HSP90-CHIP complex recognizes and selectively degrades phosphorylated tau client proteins. *J Clin Invest* **117**, 648-658.
- [26] Feuillette S, Blard O, Lecourtis M, Frebourg T, Campion D, Dumanchin C (2005) Tau is not normally degraded by the proteasome. *J Neurosci Res* **80**, 400-405.
- [27] Shimura H, Schwartz D, Gygi SP, Kosik KS (2004) CHIP-Hsc70 complex ubiquitinates phosphorylated tau and enhances cell survival. *J Biol Chem* **279**, 4869-4876.
- [28] Lee MJ, Lee JH, Rubinstein DC (2013) Tau degradation: The ubiquitin-proteasome system versus the autophagy-lysosome system. *Prog Neurobiol* **105**, 49-59.
- [29] Wang Y, Martinez-Vicente M, Kruger U, Kaushik S, Wong E, Mandelkow EM, Cuervo AM, Mandelkow E (2009) Tau fragmentation, aggregation and clearance: The dual role of lysosomal processing. *Hum Mol Genet* **18**, 4153-4170.
- [30] Simon D, Garcia-Garcia E, Royo F, Falcon-Perez JM, Avila J (2012) Proteostasis of tau. Tau overexpression results in its secretion via membrane vesicles. *FEBS Lett* **586**, 47-54.
- [31] Gomez-Ramos A, Diaz-Hernandez M, Cuadros R, Hernandez F, Avila J (2006) Extracellular tau is toxic to neuronal cells. *FEBS Lett* **580**, 4842-4850.
- [32] Kopeikina KJ, Hyman BT, Spires-Jones TL (2012) Soluble forms of tau are toxic in Alzheimer's disease. *Transl Neurosci* **3**, 223-233.
- [33] Alonso AC, Grundke-Iqbal I, Iqbal K (1996) Alzheimer's disease hyperphosphorylated tau sequesters normal tau into tangles of filaments and disassembles microtubules. *Nat Med* **2**, 783-787.
- [34] de Calignon A, Fox LM, Pitstick R, Carlson GA, Bacskai BJ, Spires-Jones TL, Hyman BT (2010) Caspase activation precedes and leads to tangles. *Nature* **464**, 1201-1204.
- [35] Farah CA, Perreault S, Liazoghli D, Desjardins M, Anton A, Lauzon M, Paiement J, Leclerc N (2006) Tau interacts with Golgi membranes and mediates their association with microtubules. *Cell Motil Cytoskeleton* **63**, 710-724.
- [36] Perreault S, Bousquet O, Lauzon M, Paiement J, Leclerc N (2009) Increased association between rough endoplasmic reticulum membranes and mitochondria in transgenic mice that express P301L tau. *J Neuropathol Exp Neurol* **68**, 503-514.
- [37] Wu JW, Herman M, Liu L, Simoes S, Acker CM, Figueroa H, Steinberg JJ, Margittai M, Kaye R, Zurzolo C, Di Paolo G, Duff KE (2013) Small misfolded Tau species are internalized via bulk endocytosis and anterogradely and retrogradely transported in neurons. *J Biol Chem* **288**, 1856-1870.
- [38] Guo JL, Lee VM (2011) Seeding of normal tau by pathological tau conformers drives pathogenesis of Alzheimer-like tangles. *J Biol Chem* **286**, 15317-15331.
- [39] Iba M, Guo JL, McBride JD, Zhang B, Trojanowski JQ, Lee VM (2013) Synthetic tau fibrils mediate transmission of neurofibrillary tangles in a transgenic mouse model of Alzheimer's-like tauopathy. *J Neurosci* **33**, 1024-1037.
- [40] Wu JW, Herman M, Liu L, Simoes S, Acker CM, Figueroa H, Steinberg JJ, Margittai M, Kaye R, Zurzolo C, Di Paolo G, Duff KE (2012) Small misfolded tau species are internalized via bulk endocytosis and anterogradely and retrogradely transported in neurons. *J Biol Chem* **288**, 1856-1870.
- [41] Gomez-Ramos A, Diaz-Hernandez M, Rubio A, Miras-Portugal MT, Avila J (2008) Extracellular tau promotes intracellular calcium increase through M1 and M3 muscarinic receptors in neuronal cells. *Mol Cell Neurosci* **37**, 673-681.
- [42] Gomez-Ramos A, Diaz-Hernandez M, Rubio A, Diaz-Hernandez JJ, Miras-Portugal MT, Avila J (2009) Characteristics and consequences of muscarinic receptor activation by tau protein. *Eur Neuropsychopharmacol* **19**, 708-717.
- [43] Saman S, Kim W, Raya M, Visnick Y, Miro S, Jackson B, McKee AC, Alvarez VE, Lee NC, Hall GF (2012) Exosome-associated tau is secreted in tauopathy models and is selectively phosphorylated in cerebrospinal fluid in early Alzheimer disease. *J Biol Chem* **287**, 3842-3849.
- [44] Andorfer C, Kress Y, Espinoza M, de Silva R, Tucker KL, Barde YA, Duff K, Davies P (2003) Hyperphosphorylation and aggregation of tau in mice expressing normal human tau isoforms. *J Neurochem* **86**, 582-590.
- [45] Yoshiyama Y, Higuchi M, Zhang B, Huang SM, Iwata N, Saido TC, Maeda J, Suhara T, Trojanowski JQ, Lee VM (2007) Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model. *Neuron* **53**, 337-351.
- [46] Spires TL, Orne JD, SantaCruz K, Pitstick R, Carlson GA, Ashe KH, Hyman BT (2006) Region-specific dissociation of neuronal loss and neurofibrillary pathology in a mouse model of tauopathy. *Am J Pathol* **168**, 1598-1607.
- [47] Kril JJ, Patel S, Harding AJ, Halliday GM (2002) Neuron loss from the hippocampus of Alzheimer's disease exceeds extracellular neurofibrillary tangle formation. *Acta Neuropathol* **103**, 370-376.
- [48] Morsch R, Simon W, Coleman PD (1999) Neurons may live for decades with neurofibrillary tangles. *J Neuropathol Exp Neurol* **58**, 188-197.
- [49] Gerson JE, Kaye R (2013) Formation and propagation of tau oligomeric seeds. *Front Neurol* **4**, 93.
- [50] Maeda S, Sahara N, Saito Y, Murayama S, Ikai A, Takashima A (2006) Increased levels of granular tau oligomers: An early sign of brain aging and Alzheimer's disease. *Neurosci Res* **54**, 197-201.
- [51] Sahara N, DeTure M, Ren Y, Ebrahim AS, Kang D, Knight J, Volbracht C, Pedersen JT, Dickson DW, Yen SH, Lewis J (2013) Characteristics of TBS-extractable hyperphosphorylated tau species: Aggregation intermediates in rTg4510 mouse brain. *J Alzheimers Dis* **33**, 249-263.
- [52] Berger Z, Roder H, Hanna A, Carlson A, Rangachari V, Yue M, Wszolek Z, Ashe K, Knight J, Dickson D, Andorfer C, Rosenberry TL, Lewis J, Hutton M, Janus C (2007) Accumulation of pathological tau species and memory loss in a conditional model of tauopathy. *J Neurosci* **27**, 3650-3662.
- [53] Diaz-Hernandez M, Gomez-Ramos A, Rubio A, Gomez-Villafuertes R, Naranjo JR, Miras-Portugal MT, Avila J (2010) Tissue-nonspecific alkaline phosphatase promotes the

- neurotoxicity effect of extracellular tau. *J Biol Chem* **285**, 32539-32548.
- [54] Plouffe V, Mohamed NV, Rivest-McGraw J, Bertrand J, Lauzon M, Leclerc N (2012) Hyperphosphorylation and cleavage at D421 enhance tau secretion. *PLoS One* **7**, e36873.
- [55] Pooler AM, Phillips EC, Lau DH, Noble W, Hanger DP (2013) Physiological release of endogenous tau is stimulated by neuronal activity. *EMBO Rep* **14**, 389-394.
- [56] Vardy ER, Kellett KA, Cocklin SL, Hooper NM (2012) Alkaline phosphatase is increased in both brain and plasma in Alzheimer's disease. *Neurodegener Dis* **9**, 31-37.
- [57] Holmes BB, Devos SL, Kfoury N, Li M, Jacks R, Yanamandra K, Ouidja MO, Brodsky FM, Marasa J, Bagchi DP, Kotzbauer PT, Miller TM, Papy-Garcia D, Diamond MI (2013) Heparan sulfate proteoglycans mediate internalization and propagation of specific proteopathic seeds. *Proc Natl Acad Sci U S A* **110**, E3138-E3147.
- [58] de Calignon A, Polydoro M, Suarez-Calvet M, William C, Adamowicz DH, Kopeikina KJ, Pitstick R, Sahara N, Ashe KH, Carlson GA, Spires-Jones TL, Hyman BT (2012) Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron* **73**, 685-697.
- [59] Liu L, Drouet V, Wu JW, Witter MP, Small SA, Clelland C, Duff K (2012) Trans-synaptic spread of tau pathology *in vivo*. *PLoS One* **7**, e31302.
- [60] Chai X, Dage JL, Citron M (2012) Constitutive secretion of tau protein by an unconventional mechanism. *Neurobiol Dis* **48**, 356-366.
- [61] Karch CM, Jeng AT, Goate AM (2012) Extracellular Tau levels are influenced by variability in Tau that is associated with tauopathies. *J Biol Chem* **287**, 42751-42762.
- [62] Bi M, Ittner A, Ke YD, Gotz J, Ittner LM (2011) Tau-targeted immunization impedes progression of neurofibrillary histopathology in aged P301L tau transgenic mice. *PLoS One* **6**, e26860.
- [63] Felder CC (1995) Muscarinic acetylcholine receptors: Signal transduction through multiple effectors. *FASEB J* **9**, 619-625.
- [64] Mizuno N, Itoh H (2009) Functions and regulatory mechanisms of Gq-signaling pathways. *Neurosignals* **17**, 42-54.
- [65] Caporaso GL, Gandy SE, Buxbaum JD, Ramabhadran TV, Greengard P (1992) Protein phosphorylation regulates secretion of Alzheimer beta/A4 amyloid precursor protein. *Proc Natl Acad Sci U S A* **89**, 3055-3059.
- [66] Hung AY, Haass C, Nitsch RM, Qiu WQ, Citron M, Wurtman RJ, Growdon JH, Selkoe DJ (1993) Activation of protein kinase C inhibits cellular production of the amyloid beta-protein. *J Biol Chem* **268**, 22959-22962.
- [67] Wang L, Shim H, Xie C, Cai H (2008) Activation of protein kinase C modulates BACE1-mediated beta-secretase activity. *Neurobiol Aging* **29**, 357-367.
- [68] Nitsch RM, Slack BE, Wurtman RJ, Growdon JH (1992) Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. *Science* **258**, 304-307.
- [69] Buxbaum JD, Oishi M, Chen HI, Pinkas-Kramarski R, Jaffe EA, Gandy SE, Greengard P (1992) Cholinergic agonists and interleukin 1 regulate processing and secretion of the Alzheimer beta/A4 amyloid protein precursor. *Proc Natl Acad Sci U S A* **89**, 10075-10078.
- [70] Haring R, Fisher A, Marciano D, Pittel Z, Kloog Y, Zuckerman A, Eshhar N, Heldman E (1998) Mitogen-activated protein kinase-dependent and protein kinase C-dependent pathways link the m1 muscarinic receptor to beta-amyloid precursor protein secretion. *J Neurochem* **71**, 2094-2103.