MODEL HIRANO BODIES DIFFERENTIALLY MODULATE CELL DEATH INDUCED BY TAU AND THE AMYLOID PRECURSOR PROTEIN INTRACELLULAR DOMAIN

by

WILLIAM EUGENE SPEARS

(Under the Direction of Marcus Fechheimer and Ruth Furukawa)

ABSTRACT

Hirano bodies are paracrystalline inclusions found in brains of patients with Alzheimer's disease (AD), frontotemporal dementia (FTD), and in normal aged individuals. Although studies of post-mortem brain tissue provide clues of etiology, the physiological function of Hirano bodies remains unknown. A cell culture model was utilized to study the role of mutant tau proteins and model Hirano bodies. Model Hirano bodies accumulate tau in human astroglioma cells. Model Hirano bodies attenuated or enhanced cell death due to tau and the amyloid precursor protein intracellular domain (AICD) depending on the form of tau. Further, model Hirano bodies enhanced or had no effect on cell death due to tau and GSK3β. These findings affirm a specific role for Hirano bodies in disease states and provides evidence that formation of Hirano bodies represents an important pathological structure in AD and FTD.

INDEX WORDS: Hirano bodies, Tau, GSK3β, Amyloid precursor protein intracellular domain, F-actin, Cell death, FTDP-17, Frontotemporal lobar degeneration, Alzheimer's disease

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DEDICATION

This thesis is dedicated to my beautiful wife, Mary. She has been an infinite source of inspiration, and a true model of excellence, determination, and open mindedness. Without her, I could not have accomplished so much. Thank you for your never-ending encouragement and wisdom.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Neurodegenerative Diseases

Neurodegenerative diseases such as Alzheimer's disease levy an overwhelming burden on modern society. For example, Alzheimer's disease (AD), the most common form of dementia, is the sixth leading cause of death in the United States (Alz.org). Further, it is the only cause of death among the top ten causes of death that cannot be effectively treated. Among neurodegenerative diseases such as AD, protein aggregations in the brain are considered major pathological hallmarks. Aggregates are often composed of proteins important for proper neuronal function such as amyloid beta (Aβ) found in senile plaques [1], the microtubule associated protein (MAP) tau found in neurofibrillary tangles (NFTs) [2], and actin and actin associated proteins that make up Hirano bodies [3]. Plaques and tangles are considered the main pathological hallmarks of AD. Much research has been focused on understanding the pathogenesis of AD with limited success, and the contribution of aggregations themselves to pathogenesis of disease is widely debated (for review, see [4, 5]). Common pathways in Alzheimer's disease and other neurodegenerative diseases result in accumulation of insoluble inclusions, cognitive decline, and massive synapse and neuron loss [6].

Amyloid precursor protein

The amyloid precursor protein (APP) is a transmembrane protein subject to multiple cleavage events mediated by three proteases; α, β, and γ-secretase [7-9]. APP

proteolysis by β and γ-secretase releases an extracellular peptide known as Aβ. Intramembrane proteolysis of APP by γ-secretase also releases an intracellular fragment known as the amyloid precursor protein intracellular domain (AICD). AICD can be further processed by caspases that cleave the C-terminus of APP to release a 31 amino acid peptide called c31 [10, 11]. The amyloid cascade hypothesis suggests that Aβ is the primary cause of neurodegenerative alterations in AD, and accordingly, Aβ has been the central target of basic research and therapeutics in AD. Despite this, Aβ-directed therapies have often failed in the clinic, bringing to light new evidence that AICD may play an important, understudied role in neurodegenerative disease pathways. AICD has been shown to be imported into the nucleus where it activates transcription of target genes and causes cell death [12-15]. Although consistent identification of target genes remains elusive, it is known that AICD is capable of forming a transcriptionally active complex composed of the histone acetyltransferase Tip60 and the adaptor protein Fe65, or the transcription factor CP2/LSF/LBP1 [16-20]. It has recently been shown that AICD+Fe65 over-expressing mice exhibit AD-like phenotypes including increased levels of hyperphosphorylated and insoluble tau and cognitive decline not caused by Aβ [21-24]. Expression of both transgenes probably plays a critical role since either Fe65 or AICD single transgenic mice fail to cause AD-like phenotypes. Interestingly, Fe65 has been reported to colocalize with NFTs found in AD [25] and has also been shown to bind tau directly [26]. It is also able to bind mena [27], an actin binding protein, suggesting a possible link between the actin and microtubule cytoskeleton. Although controversial, multiple studies have reported that a mutation in the C-terminus of APP (D664A) that

prevents caspase cleavage and release of c31 rescued neurodegeneration induced by mutant APP [28-30].

Tau

Tau is a microtubule associated protein known to bind, stabilize, and promote microtubule assembly in vitro [31, 32]. A single MAPT gene generates six tau isoforms by alternative splicing of its pre-mRNA (Figure 1). Isoforms ranging in size from 352-441 residues differ by exclusion or inclusion of exons 2,3, and 10. This results in the generation of either three or four C-terminal microtubule binding repeats (called 3R or 4R) and zero, one, or two N-terminal repeats (called 0N, 1N, or 2N). Hence, 441 length tau is denoted 4R2N tau. Although the importance of tau expression is debated, it may be needed for proper axonal transport [33, 34], growth cone maintenance [35], microtubule stability [36-39], iron export [40], DNA damage repair [41], and post-synaptic receptor localization [42]. Tau is an inherently unfolded, highly flexible protein, and because of this is subject to a variety of post-translational modifications that regulate its function [43]. The most studied of these modifications is phosphorylation due to the fact that hyperphosphorylated tau is the primary component of neurofibrillary tangles (NFTs) found in Alzheimer's Disease, frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17), and other neurodegenerative conditions [44-46]. It is thought that post-translational modification of tau at specific residues facilitates a toxic gain of function driving the formation of soluble species or insoluble aggregates that promote cell death [42, 47, 48]. However, identification of the precise mechanisms and pathological tau species remains elusive. Self-aggregation of tau is mediated by its microtubule binding repeats, and the presence of N-terminal or C-terminal regions of tau

generally inhibits aggregation. However, specific modifications such as phosphorylation can, in some cases, promote aggregation of full-length tau [49]. Tau is thought to be essential to cell death pathways in AD and FTDP-17, and reduction of tau in cell culture and mouse models of AD prevents cytoskeletal breakdown, neuronal loss and behavioral impairments induced by Aβ [50-53]. According to the amyloid cascade hypothesis, Aβ acts upstream of tau, inducing tau hyperphosphorylation and cell death [54-57].

Tauopathies

The term "tauopathy" refers to a variety of conditions characterized by the formation of insoluble aggregates of hyperphosphorylated tau [58]. These conditions include Alzheimer's disease as well as many conditions in which tau accumulation is the primary pathological lesion, such as frontotemporal lobar degeneneration with tau inclusions (FTLD-tau) [59]. Many of these conditions occur sporadically such as in progressive supranuclear palsy (PSP), corticobasal degeneration (CBD), and Pick's disease (PiD)(for review, see [60]). These conditions are mainly characterized by tau polymerization, neuron loss, and gliosis in the frontal and temporal cortices. However, subtle differences between these diseases include differences in the structure of tau deposits, in their ratio of 3R/4R tau, and involvement of non-neuronal cell types. For example, PSP is characterized by glial fibrillary tangles in astrocytes and oligodendrocytes [61-63]. Biochemically, tangles found in PSP constitute two high molecular weight bands immunoreactive to hyperphosphorylated tau antibodies, rather than the three typically found in AD brains [64, 65]. In contrast, CBD is pathologically characterized by extensive neuropil threads, or tau inclusions present in the neurite-rich

areas of the brain [66, 67]. In contrast to PSP, tau inclusions in CBD are composed entirely of 4R tau isoforms [68].

Autosomal dominant mutations in tau cause another group of tauopathies called frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) [69-71]. Nearly 40 mutations in tau cause dementia in this disease, many of which are point mutations located in or near the microtubule binding domain [72]. Many of these mutations have been shown to alter the biochemical properties of tau, notably its ability to bind and promote microtubule assembly (Table 2). Some of these mutants have also been shown to increase the tendency of tau to aggregate, probably through modulation of PHF6 motifs in tau shown to be important for polymerization [73-76]. Other mutants promote disease progression by altering the alternative mRNA splicing of exon 10 (MT binding repeat two) resulting in increased expression of 3R tau [60]. This alters the 1:1 ratio of 3R/4R tau thought to be important for proper neuronal function, resulting in decreased microtubule stability, axonopathy, and buildup of tau deposits [77]. Disease causing tau mutations show differential patterns of tau deposition, age of onset, duration, and clinical symptoms such as parkinsonism, personality change, speech disorders, and language difficulties [72] (Table 2). This underscores the importance of differential losses and gains of function of each tau mutant. More work is needed to better understand the normal function of tau as well as important gains or losses of function caused by mutations that may contribute to neurodegeneration.

Tauopathies including FTDP-17 are often commonly noted as widely devoid of other pathological inclusions such as amyloid beta containing plaques. However, recent evidence suggests that seemingly disparate neurodegenerative diseases may be more

commonly connected than previously thought. For example, TAR DNA-binding protein 43 (TDP43) was recently identified as the major protein component of amyotrophic lateral sclerosis (ALS) and a subtype of FTLD now known as FTLD-TDP [78]. This discovery led researchers to discover that TDP43 positive inclusions are a significant finding in a variety of diseases such as AD, primary tauopathies, and Lewy body disorders [79, 80]. More studies are needed to determine whether TDP43 plays an important role in neurodegeneration that occurs in these other conditions.

In addition to TDP43, amyloid beta containing plaques are not thought to play a role in the pathogenesis of FTDP-17 as tau deposits are considered the main pathological finding. However, multiple studies show diffuse or mature plaque pathology in FTDP-17 patient brains. Plaque pathology has been reported in patient brains harboring either an R5H, ΔK280, or P301L tau allele [81-85]. It remains a question why plaque pathology may take a backseat to predominant tangles, but it may implicate abnormal processing of APP in the pathogenesis of FTDP-17.

Hirano Bodies

Hirano Bodies are another pathological hallmark of aging and neurodegeneration that link a wide variety of conditions. Hirano bodies were originally discovered by Dr. Asao Hirano in patients with amyotrophic lateral sclerosis and parkinsonism-dementia complex (ALS-PDC) on Guam [86]. They are eosinophilic, paracrystalline inclusions composed largely of F-actin [87, 88], and they accumulate a variety of cytoskeletal proteins and signaling molecules (summarized in [89]). Hirano bodies are found in brains of normal aged individuals [90, 91], and are characteristic of neurodegenerative diseases such as Alzheimer's disease [92, 93], amyotrophic lateral sclerosis and Parkinson's

disease [86], tauopathy including FTLD-tau and FTLD-TDP [94], prion diseases such as Creutzfeldt-Jakob disease [94, 95] and chronic wasting disease [96, 97], diabetes [98], and chronic alcoholism [99]. Hirano bodies or Hirano body-like structures (HBLS) have also been reported in animal models of AD and tauopathy including those expressing mutant forms of APP, tau, or both [100-103]. For example, HBLS were reported in Drosophila brains expressing exogenous FTDP-17 tau, where they facilitated retinal degeneration [103]. In the same report, HBLS were also observed in P301L tau transgenic mice. Other studies report that neuronal stress facilitates dramatic re-arrangement of F-actin into inclusions reminiscent of but structurally distinct from Hirano bodies [104, 105]. Interestingly, these inclusions known as actin-cofilin rods (AC rods) accumulate hyperphosphorylated tau [106].

Due to lack of a model system to study Hirano bodies, most studies to date focus on analysis of post-mortem material. These reports show that Hirano bodies accumulate a variety of epitopes including tau and other MAPs [89, 103, 107, 108], C-terminus of APP [13, 109], and other actin associated proteins [89]. Interestingly, NFTs were often reported to be intermingled in the same cell as Hirano bodies, suggesting a possible connection [86]. In this study, Hirano, et al. found a rough positive correlation between the frequency of NFTs and Hirano bodies. Similar to NFTs and Pick bodies, Hirano bodies are frequently found in the CA1 region of the hippocampus although they are rarely reported elsewhere [110]. Hirano bodies have been reported in a variety of subcellular localizations in neuronal and non-neuronal cells. However, they are most often reported in axons and dendritic processes [110]. Some reports identify Hirano bodies in the soma [87], and still others identify Hirano bodies in oligodendrocytes [92],

schwann cells [111], and in the perineuronal space typically occupied by satellic glial nuclei [86, 111, 112]. Reports also show the appearance of Hirano bodies as early as the second decade of life, before the presence of other neuropathological changes [113].

Hirano bodies possess a distinct ultrastructure, and depending on the plane of section may appear as intersecting parallel filaments, or as a straight row of punctate densities attributed to F-actin [93, 114]. These filaments are parallel bundles of F-actin that also appear to be stacked in uneven strata. Hirano bodies are structurally distinct from AC rods, which are long, narrow bundles of F-actin saturated with ADF/cofilin found throughout the neuropil in AD brains [115]. AC rods are immunoreactive towards anti-actin antibodies, but unlike Hirano bodies are not stained with phalloidin. More studies are needed to determine if Hirano bodies and AC rods share similar mechanisms of formation, and their existence further underscores the importance of re-arrangement of actin in aging and neurodegeneration.

Model system for the formation of Hirano bodies

We have developed a model system to study Hirano bodies through expression of CT, a truncated form of 34-kDa protein, an actin bundling protein found in Dictyostelium discoideum [89, 116-120]. 34-kDa protein is an actin bundling protein that saturates F-actin binding at a ratio of 1 34-kDa monomer to 10 actin monomers in a filament [121]. It has a reduced affinity for actin at micromolar concentrations of calcium and millimolar concentrations of magnesium [121]. 34-kDa protein localizes to filopodia, cortical regions, central cytoplasm, posterior regions of polarized cells, and is enriched at sites of cell-cell contact [121-123]. 34-kDa null Dictyostelium grow and divide normally, but exhibit some deficits in motility. These cells displayed a higher persistence of motility,

and increased length and number of filopodia [124]. These phenotypes are likely due to some redundancy between other actin bundling proteins

Protein truncation analysis of 34-kDa protein fragments revealed a total of three actin binding sites: a strong actin binding site (a.a. 193-254), an N-terminal binding site (a.a. 1-123), and a C-terminal basic region (a.a. 279-295) [125]. Further analysis also revealed the presence of two intramolecular interaction zones in 34-kDa protein, denoted IZ-1 and IZ-2. Because truncations composed of C-terminal regions of 34-kDa protein bind F-actin with a higher affinity, it was proposed that the N-terminal region of 34-kDa protein functions as an inhibitory domain by interacting with the strong actin binding site, resulting in slightly weaker actin binding. In support of this, mutations in the IZ-1 domain of 34-kDa protein result in strong, calcium insensitive F-actin binding similar to CT [119].

Truncation of 34-kDa to form CT (C-terminus, a.a. 124-295) revealed activated actin binding activity that was calcium insensitive [119, 125]. This fragment contains the second of two putative calcium binding EF hands and is missing the N-terminal region. In multiple cell lines including HeLa, Cos7, HEK293, neuronal and glial cell lines, as well as in primary neurons, CT expression resulted in ellipsoid shaped inclusions identified as model Hirano bodies [116, 117]. Similar to authentic Hirano bodies found in aged brain tissue, model Hirano bodies strongly accumulate CT, F-actin, actin associated proteins, transcription factors, and other signaling molecules [89]. Model Hirano body formation did not induce cell death and had only minor effects on cell growth [89, 116]. Other mutants of 34-kDa protein have since been shown to induce model Hirano body formation. ΔEF1 results from mutation of three aspartate residues to alanine in the first

putative EF hand [117]. Although EF2 was shown to be the only putative EF hand able to bind calcium, ΔEF1 resulted in strong calcium insensitive actin binding activity and Hirano body formation. This is probably because ΔEF1 disrupts IZ-1, interfering with the interaction of the N-terminal inhibitory domain with the strong actin binding site. Inhibition of calcium binding alone does not result in activated actin binding activity since the same aspartate to alanine mutations in EF2 (ΔEF2) results in loss of calcium binding activity of 34-kDa protein but does not result in HB formation [117].

Recently, we have created a mouse model for the formation of model Hirano bodies, resulting in expression of CT-GFP in the hippocampus and cerebral cortex of $Thy1Cre^{+/+}CT^{+/+}$ mice [126]. CT-GFP was highly expressed in the CA3 region of the hippocampus, and Hirano bodies were frequently identified as eosinophilic, cytoplasmic inclusions in the brains of 6 month old homozygous mice. These animals were viable and no gross defects were observed compared to wild type controls. However, electrophysiological studies of brain slices from model Hirano body mice showed a strong paired pulse depression, and deficits in early long-term potentiation although no deficits in LTP were observed compared to wild type controls 240 min. post-tetanus. These results further suggest that the presence of Hirano bodies are not deleterious, although formation of Hirano bodies may cause some slowing of vesicular trafficking at the synapse, and delayed trafficking of NMDA and AMPA receptors during early LTP [126].

Figure 1: *Alternative splicing of tau.* Adapted from [127]. Six isoforms of tau are generated from alternative splicing of its pre-mRNA. All isoforms originate from a single gene located on chromosome 17. Alternative splicing of exons 2, 3, and 10 results in the presence of either 0, 1, or 2 N-terminal repeats (0N, 1N, or 2N), and the presence of either 3 or 4 C-terminal microtubule binding repeats (3R or 4R). Thus, the shortest tau isoform is 352 amino acids in length (0N3R), and the longest tau isoform is 441 amino acids in length (4R2N).

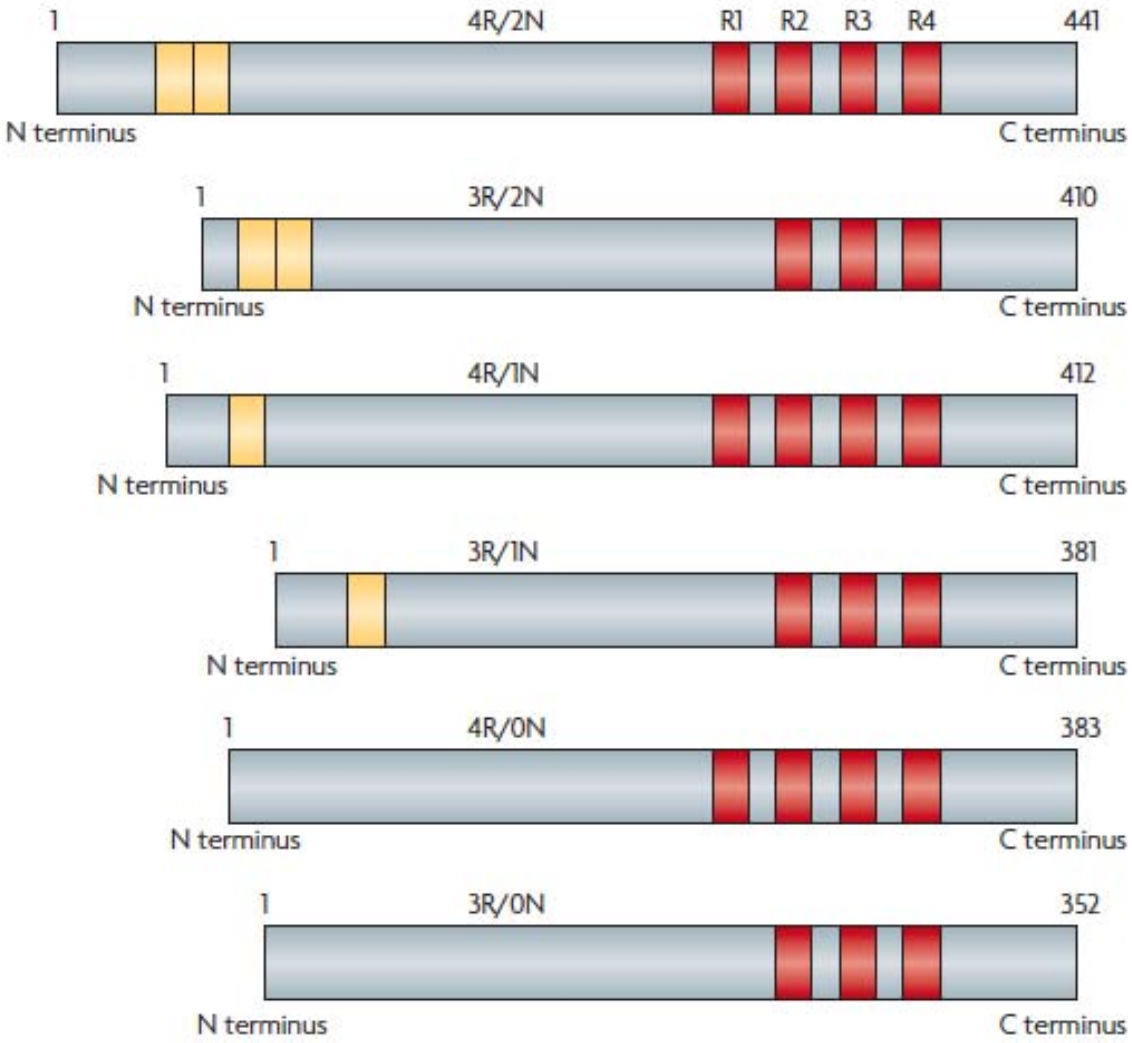

1
4R/2N
R1
R2
R3
R4
441
N terminus
C terminus
1
3R/2N
410
N terminus
C terminus
1
4R/1N
412
N terminus
C terminus
1
3R/1N
381
N terminus
C terminus
1
4R/0N
383
N terminus
C terminus
1
3R/0N
352
N terminus
C terminus

Table 1. Evidence of tau-actin interactions		
Assay	***Result***	***Reference***
Viscosity	MAPs increase viscosity of actin, actin+tubulin mixtures	[128]
Viscosity	Critical gelation concentration of mixtures of actin and MAPs increased with increased phosphorylation	[129]
Viscosity, turbidity, TEM	Tau assembles F-actin, increases turbidity, bundles actin	[130]
Viscosity	Phospholipids have no effect on viscosity of tau-actin mixtures	[131]
ELISA, TEM	Actin inhibits tau-tubulin interactions. Tau forms actin bundles and is observed at cross-links	[132]
TEM, cosedimentation, affinity chromatography	MT binding domain tau peptide and full length tau bind actin, only full length tau bundles actin	[133]
Cosedimentation, TEM, AFM	Tau MT binding domain or proline-rich region sufficient for actin binding	[134]
Overexpression of FTDP-17 tau in Drosophila	FTDP-17 tau expression causes phospho-tau reactive actin inclusions	[103]
Primary chick neuron culture, colocalization	Cell stress induces phospho-tau accumulation in actin-cofilin rods	[106]
N2A cell culture; human brain immunohistochemistry	Endogenous tau colocalizes with HBs in culture and in AD	[89, 107, 108]
NGF treated PC12 cells, colocalization	Tau colocalizes with actin in lammellipodia	[135]
PC12 cell culture	Interaction of tau with the plasma membrane dependent on F-actin	[136, 137]

Table 2. Tau mutations produce different phenotypes			
Tau mutant	***Method***	***Result***	***Reference***
R5H	Genomic DNA sequencing, MT assembly assay	Patient with tau R5H allele shows NFTs, plaques. Recombinant R5H shows reduced ability to promote MT Assembly	[82]
G272V	Overexpression of 441 G272V human tau driven by prion protein promoter	Oligodendroglial hyperphosphorylated tau filaments, reduced tau solubility	[138]
G272V	Clinical symptoms	Onset 41-50 yrs, duration 6-10 yrs, late dementia, parkinsonism rare, early personality change, language difficulties	[72]
G272V, P301L, ΔK280	Live cell tracking of GFP-tubulin+Tau transfected MCF7 cells	G272V tau reduced ability to regulate MT dynamics	[139]
P301L, R406W	Light scattering, cosedimentation, frontal gray matter tau exraction	Reduced ability to bind and promote MT assembly. R406W had lowest MT binding capacity. Human P301L insoluble tau composed of 4R. R406W composed of all 6 isoforms	[140]
P301L	Clinical symptons	Onset 41-50, duration 6-10 yrs, rare parkinsonism, late dementia, early personality change, language difficulties, amyotrophy	[72]
P301L	4R0N or 4R2N P301L expression in mice	NFT formation, neuron loss, age-dependent increase in insoluble tau, tau filament formation, astrocyte activation	[141, 142]
P301L	CamKII driven 4R0N mouse tau expression, primary neuron culture	Mice show memory and synaptic plasticity deficits, tau accumulation in dendritic spines, decreased synaptic expression of AMPA and NMDA subunits	[48]
R406W	CamKII driven forebrain expression of 4R2N	Tau inclusions, filament formation, memory deficits. All tau isoforms expressed were incorporated in tangles	[143]
R406W, P301L	Primary neuron culture	Wt and R406W tau expression potentiated cell death in the presence of APP, not P301L	[47]
R406W	Clinical symptons	Onset >50 yrs, duration >15 yrs, early dementia, personality change, mutism	[72]
R406W	Primary neuron culture	R406W decreases phosphorylation, abolishes tau interaction with the plasma membrane mediated by Annexin A2	[144]
ΔK280	Genomic DNA sequencing, IHC	NFTs, preferential 3R tau deposits, may increase splicing of 3R tau	[81]
K18ΔK280	CaMKII driven expression	Mice show hyperphosphorylation of endogenous mouse tau, neuron loss, NFTs, tau mislocalization	[145]
K18ΔK280	N2a cell culture	Binds weakly to MTs, high propensity for aggregation which is correlated with further proteolysis and cell death	[75, 146, 147]

CHAPTER 2

HIRANO BODIES DIFFERENTIALLY MODULATE CELL DEATH INDUCED BY TAU AND THE AMYLOID PRECURSOR PROTEIN INTRACELLULAR DOMAIN[1]

[1] William Spears, Marcus Fechheimer, Ruth Furukawa. To be submitted to BMC Neuroscience, 2012.

Abstract

Hirano bodies are paracrystalline inclusions found in brains of patients with Alzheimer's disease (AD), frontotemporal dementia (FTD), and in normal aged individuals. Although studies of post-mortem brain tissue provide clues of etiology, the physiological function of Hirano bodies remains unknown. A cell culture model was utilized to study the role of mutant tau proteins and model Hirano bodies. Model Hirano bodies accumulate tau in human astroglioma cells. Model Hirano bodies attenuated or enhanced cell death due to tau and the amyloid precursor protein intracellular domain (AICD) depending on the form of mutated tau. Further, model Hirano bodies enhanced or had no effect on cell death due to tau and GSK3β. These findings affirm a specific role for Hirano bodies in disease states and provides evidence that formation of Hirano bodies represents an important pathological structure in AD and FTD.

Introduction

The cause of sporadic Alzheimer's disease (AD) is unknown, and an intricate interaction between multiple genetic, epigenetic, and environmental risk factors have been proposed (for review, see [6]). However, in roughly 1% of total AD cases, studies show that neurodegeneration results from mutations in the genes encoding the amyoid precursor protein (APP), presenilin 1 (PSEN1), or PSEN2 [148, 149]. These mutations result in altered processing of APP, increased deposition of amyloid beta (Aβ), and early onset neurodegeneration. This led to the formation of the amyloid cascade hypothesis, in which it is thought that Aβ inititates a cascade of events leading to tau deposition,

synaptic dysfunction and cognitive decline [54]. Proteolysis of APP by β and γ-secretase releases Aβ. Intramembrane proteolysis of APP by γ-secretase also releases an intracellular fragment known as the amyloid precursor protein intracellular domain (AICD). AICD has been shown to form multi-protein complexes that regulate the induction of apoptosis (for review, see [150, 151]). Multiple studies suggest that a downstream effector of AICD is the kinase GSK3β. Studies in neuronal cell cultures and in vivo report upregulation of total levels of GSK3β as well as increased activation, and tau hyperphosphorylation [18, 21, 152].

Complementary to the amyloid cascade hypothesis was the discovery that mutations in tau cause a group of familial neurodegenerative diseases called frontotemporal dementia with parkinsonism linked to chromosome 17 (FTDP-17) [153]. These diseases are characterized pathologically by the formation of neurofibrillary tangles (NFTs) composed of hyperphosphorylated tau [60]. Aβ containing plaques found in AD are often not considered a significant pathological feature of FTDP-17. Thus, the amyloid cascade hypothesis posits that Aβ acts upstream of tau to promote neurodegeneration. Nevertheless, significant plaque pathology is often reported in FTDP-17 patient brain tissue [81-85].

Hirano bodies are inclusion bodies found in significant numbers in the hippocampus of patients with AD compared to age matched controls [91]. In addition, Hirano bodies are found in post-mortem brain tissue of patients afflicted with a variety of neurodegenerative and other diseases, and in normal aged individuals [110]. Hirano bodies are also found in patients diagnosed with FTLD-tau such as PSP and PD [154], although no studies to date explore the cellular basis of this. Although the contribution of

Hirano bodies to FTDP-17 is unknown, cell culture and transgenic animal models of FTDP-17 suggest that alterations in the actin cytoskeleton and formation of Hirano bodies are an important event in the pathogenesis of tauopathy [103, 104, 106]. Hirano bodies are thought to be composed of primarily filamentous actin and actin associated proteins [3, 88, 155], but they have also been shown to contain other components of the neuronal cytoskeleton such as the middle molecular weight neurofilament subunit [113] and the microtubule associated protein (MAP) tau [107, 108]. Hirano bodies also accumulate a number of signaling proteins including the transcription factor FAC1 [156], transforming growth factor-β3 [157], and AICD [13, 109]. Interestingly, Hirano, et al proposed a positive correlation between the frequency of NFTs and Hirano bodies in patient samples [86]. In this study, Hirano bodies and NFTs were often found in the same cell. Although these reports suggest that tau colocalizes with Hirano bodies, the specific isoforms, mutants, or modifications on tau and physiological consequences of this are unknown.

A single MAPT gene generates six tau isoforms by alternative splicing of its pre-mRNA. Isoforms ranging in size from 352-441 residues differ by exclusion or inclusion of exons 2,3, and 10. Tau is a microtubule associated protein localized mainly in neuronal axons [158], and is also found in oligodendrocytes [159]. In both cell types, hyperphosphorylated tau is thought to play an important role in the neurodegeneration since it is the main component of NFTs [44, 160, 161]. Additionally, abnormal tau phosphorylation is an early marker of AD [162, 163] and phosphorylated tau levels correlate with the severity of AD [164]. However, the precise mechanisms causing abnormal tau phosphorylation leading to pathological tau formation remain unclear.

Early studies of Hirano bodies were reliant on immunohistochemical staining of post-mortem brain tissue due to lack of a model system to study their formation or physiological function. We have developed a cell culture model of Hirano bodies by expressing a truncated form of 34-kDa protein from *Dictyostelium* [89, 116-119]. Truncation of the amino terminus of 34-kDa protein to form CT results in a gain of function in activated actin binding activity and formation of model Hirano bodies in mammalian cells and in transgenic mice [117, 126]. Model Hirano bodies mitigate the transcriptional activating activity of the APP intracellular domain (AICD), resulting in a decrease in cell death [13]. Further, model Hirano bodies decrease cell death potentiated by AICD and a pseudohyperphosphorylated (PHP) tau mimic [165].

We investigated the association of both wild type and mutant forms of tau with Hirano bodies, and the impact of this on cell death pathways involving tau and AICD. We report that all tau isoforms and mutants tested associate with model Hirano bodies. However, tau mutants differentially influenced cell death in the presence/absence of AICD and model Hirano bodies. Additionally, we show that model Hirano bodies potentiate cell death initiated by tau and GSK3β. These results extend previous reports affirming a specific role for Hirano bodies in aging and disease states and provides further evidence that the presence of Hirano bodies may have an important contribution to the pathogenesis of AD and FTD.

Methods

Cell culture and expression plasmid construction

Human H4 astroglioma cells (American Type Culture Collection, Manassas, VA) were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10%

fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA) at 37°C and 5% CO_2. Transient transfection was performed with either FuGene 6 (Roche Applied Science, Nutley, NJ) or Lipofectamine LTX (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. For generation of an expression construct for model Hirano body formation, C-terminal amino acids 124-295 from *Dictyostelium* were cloned into the BamH1 site of pEGFP-N1 (Clontech, Mountain View, CA) to generate the CT-GFP fusion protein [89]. 352PHP is a tau mutant created in the shortest (352) tau isoform in which 10 serine/threonine residues (S198, S199, S202, T231, S235, S396, S404, S409, S413, and S422) were mutated to glutamic acid to mimic a hyperphosphorylated state (a generous gift from Roland Brandt, University of Osnabrück, Osnabrück, Germany) [166]. To generate N-terminal FLAG-tagged tau constructs, the coding sequence of 352PHP was digested from the 352PHP plasmid with ClaI. The coding sequence of 441 length human wt tau was digested out of pET-29b (Addgene plasmid 16316) [167]. ClaI restriction sites were introduced through PCR, and the tau coding sequence was cloned into the ClaI site of the remainder of the 352PHP plasmid backbone. Tau mutants were constructed from 441 length tau using a QuikChange II XL Site-Directed Mutagenesis Kit (Stratagene, La Jolla CA). Primers for mutagenesis were:

ΔK280 5' GCAGATAATTAATAAGCTGGATCTTAGC,

R5H 5'ATGGCTGAGCCCCACCAGGAGTTCGAAG,

G272V 5'GCACCAGCCGGGAGTCGGGAAGGTGCAG,

P301L 5'ATCAACACGTCCTGGGAGGCGGCAG,

R406W 5'GGACACGTCTCCATGGCATCTCAGCAATG. Tau constructs K18 and K18ΔK280 comprise the microtubule binding repeats of 441 length tau (a.a. 244-372), and were generated using the following primers:
sense 5' CGATAGATCGATGCAGACAGCCCCCGTGCCCATGCC,
anti-sense 5' CGATAGATCGATTCATTCAATCTTTTTATTTCCTCCG. ClaI sites were added to each end of K18 through the above primers and PCR products were digested and ligated into the ClaI site of the remainder of the 352PHP plasmid backbone after deletion of the coding sequence. Coding sequences of all plasmids were verified by sequencing.

Cell death assays

24 hours prior to transfection, 8,000 H4 cells/well were plated into 96-well plates (Nalge Nunc, Rochester, NY). Cells were transiently transfected with equal amounts of plasmid (250 ng each) using Lipofectamine LTX (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Plasmids encoded GFP (pEGFP-N1, Clontech, Mountain View, CA), CT-GFP [89], AICD (APPc58-myc, C-terminal 58 amino acids of APP-695, a generous gift from Bradley Hyman, Harvard Medical School) [168], or HA epitope-tagged GSK3β (Addgene plasmid 15994) [169]. 24 hours post-transfection, H4 cells were incubated with a mixture of a final concentration of 264 μM Hoechst 33258 and 9 nM Sytox Orange (Molecular Probes) for 15 min prior to visualization using a Zeiss IM-35 epifluoresence microscope with a 63x oil immersion objective equipped with a CCD 300-T-RC camera (Dage MTI) controlled by Scion Image software. Fluoresence images from GFP, Sytox Orange, and Hoechst was superimposed in Photoshop and live and dead cells were counted. Since H4 cells have low transfection efficiencies (~30%), the presence of

EGFP or CT-GFP was used to indicate transfected cells. The total number of GFP positive, sytox orange positive cells was compared to total GFP positive cells and displayed as percent cell death. At least three independent trials were conducted per experiment, and > 50 cells/well were counted for each trial. Mean and standard deviations are plotted in the graphs. Analysis of statistical significance was performed using Student's t-test.

Immunofluoresence

H4 cells were plated onto glass coverslips and allowed to adhere for 24 hours. Cells were transiently transfected with equal amounts of plasmid (1 μg each) using Lipofectamine LTX (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. 24 hours post-transfection, cells were fixed in 3.7% formaldehyde in PBS for 10 min, washed three times for 10 min each in PBS, permeabilized with 0.2% Triton X-100 for 5 min, and blocked with 5% bovine serum albumin in PBS for 1 h at room temperature. Cells were incubated with appropriate primary antibody for 1 h at rom temperature, washed 3 times 10 min each in PBS and incubated with secondary antibody for 1 h at room temperature. Cells were washed 3 times 10 min in PBS and mounted on glass slides using Crystal Mount (Biomedia). Coverslips were visualized with a Zeiss Axioobserver Z1 equipped with an AxioCam MRm controlled by AxioVision4.6 software. Antibodies used were anti-FLAG rabbit antibody to label tau (Sigma-Aldrich, St. Louis, MO), and Alexa Fluor 350 conjugated anti-rabbit secondary antibody (Molecular Probes, Eugene, OR). F-actin was visualized using TRITC conjugated phalloidin (Sigma-Aldrich, St. Louis, MO), and nuclei were stained with DAPI (Sigma-Aldrich, St. Louis, MO).

Results

Localization of tau and Hirano bodies

The interaction of altered forms of tau with model Hirano bodies and the impact of this interaction on disease pathways leading to cell death was investigated in this study. Hirano bodies have been proposed to have a glial origin [92], and because glial cells have been shown to be important mediators of cell death induced by Aβ and tau [170], H4 human astroglioma cells were utilized.

Since some studies show differences in the biochemical properties of FTDP-17 mutated tau [74, 75, 82, 139, 140, 171], and because preferential accumulation of different forms of tau might indicate physiological function of Hirano bodies, mutant tau and model Hirano body localization was investigated. FLAG-tagged wt tau expression plasmids were created in the longest (441 a.a.) and shortest (352 a.a.) isoforms, and tau mutants R5H, G272V, P301L, and R406W were created in 441 length tau (Figure 1A). We also utilized a FLAG-tagged tau mutant that mimics a hyperphosphorylated state through mutagenesis of 10 serine/threonine residues to glutamate (352PHP) [166], and a tau mutant known to aggregate in cell cultures (K18ΔK280) [146]. All mutant tau proteins as well as wt tau isoforms showed strong colocalization with model Hirano bodies except for 352PHP, which was diffusely localized throughout the cell, as shown in Figure 1B. Further, truncated tau mutants containing only the microtubule binding region of tau (K18 or K18ΔK280) colocalize with model Hirano bodies. These two mutants also showed a strong nuclear localization. In contrast, all other tau constructs were cytoplasmic (Figure 1B). In general, tau appeared to be present throughout the model Hirano body or formed a ring-like structure around the periphery of the model Hirano

body. These results show that regardless of specific point mutations, tau shows enrichment at sites of F-actin accumulation. However, hyperphosphorylation at may decrease this association, as shown for 352PHP tau.

Hirano Bodies, tau, and AICD

In order to functionally assay the effects of disease relevant tau protein, Brandt and colleagues developed a tau phosphomimic (352PHP) [136, 166, 172-176]. Mutated residues represent sites of hyperphosphorylation identified in paired helical filaments (PHFs) of AD patients, which are precursor structures to mature NFTs [177]. Previous reports show that PHP tau induces cell death when expressed in neuronal cell cultures [47, 166, 173]. Model Hirano bodies protected against AICD-induced cell death in the presence of 352 wt tau or 352PHP tau [165]. We investigated whether model Hirano bodies would have an effect on cell death induced by wt tau isoforms and tau mutants found in FTDP-17 in the presence of AICD. As previously reported, expression of AICD resulted in modest levels of cell death (figure 2A, white bars), and model Hirano bodies significantly lowered this death (white bars $*p < 0.05$). Transfection of 352 or 441 wt tau resulted in low levels of cell death (gray bars), and the presence of model Hirano bodies had no effect on this cell death (check bars). Although cotransfection of wt tau isoforms+AICD (black bars) caused an incremental increase in cell death, a marked potentiation in cell death was observed upon cotransfection of 352PHP+AICD consistent with previous data from our laboratory [165]. The presence of model Hirano bodies protected against cell death induced by both 352 wt tau+AICD ($***p < 0.001$), 441 wt tau+AICD ($**p < 0.01$), and 352PHP tau+AICD (stripe bars, $** p < 0.01$). These results

suggest that tau hyperphosphorylation enhances cell death and that model Hirano bodies are able to mitigate cell death induced by wt and hyperphosphorylated tau.

FTDP-17 tau and Hirano bodies differentially modulate cell death.

Previous studies indicate that expression of FTDP-17 tau has differential cytototoxic effects when expressed in cell cultures and in various animal models of tauopathy [42, 48, 103] that may be attributable to the biochemical properties of tau [73, 74]. Given this, the effect of mutated tau on AICD-induced cell death in the presence or absence of model Hirano bodies was investigated. As expected, exogenous expression of tau mutants (gray bars) R5H (figure 2B), R406W (figure 2B), G272V (figure 2C), or P301L (figure 2C) did not produce significant cell death. Similarly, coexpression of either R5H or R406W tau in the presence of model Hirano bodies resulted only in background levels of cell death (figure 3B, check bars). In contrast, expression of either G272V tau (**$p < 0.01$) or P301L (***$p < 0.001$) tau in the presence of model Hirano bodies resulted in significant potentiation of cell death (figure 2C, check bars). Similar to 352PHP tau, FTDP-17 tau mutants enhance cell death in the presence of AICD compared to wt tau isoforms+AICD (figure 2B,C, black bars). The presence of model Hirano bodies protected from cell death due to AICD and either R5H or R406W tau (Figure 2B, stripe bars **$p < 0.01$). However, in cells expressing either G272V tau+AICD or P301L tau+AICD, model Hirano bodies had no effect on cell death (figure 2C, stripe bars). These data suggest that model Hirano bodies either mitigate or promote cell death induced by tau and/or AICD, depending on the tau variant used.

Hirano bodies are not protective against aggregation prone tau

The tau mutant ΔK280 is representative of a group of FTDP-17 mutants previously shown to have increased susceptibility to aggregation compared to wt tau [74-76], although whether or not tau polymerization is directly neurotoxic continues to be a major question (for review, see [4]). It has been demonstrated that different stages of tau aggregates pose disparate consequences to cells ranging from toxic in the oligomer form [178] to inconsequential or protective in the mature NFT form [179]. Previous studies have established a cell culture model of tau aggregation through expression of a tau fragment with a high propensity for β-structure and aggregation [147], termed K18ΔK280. Studies show that expression of K18ΔK280 tau causes cell death prior to the formation of mature aggregations [145-147]. The effect of K18ΔK280 tau on cell death in the presence of model Hirano bodies was investigated. Consistent with previous reports, expression of K18ΔK280 resulted in significant cell death compared to expression of K18 (figure 2D, gray bars ***$p < 0.001$). Model Hirano bodies had no effect on this death (check bars). Expression of K18ΔK280 with AICD increased cell death (black bars *$p < 0.05$), and the presence of model Hirano bodies lowered this cell death (stripe bars *$p < 0.05$). Notably, CT-GFP expression only lowered levels of cell death due to AICD.

Hirano bodies differentially influence cell death induced by GSK3β and tau.

GSK3β is one of the most well studied kinases in AD, and its contribution to pathogenesis of AD is likely essential (for review, see [180]). Specifically, GSK3β is thought to become upregulated and over-activated by Aβ and AICD, triggering a cascade of signaling events leading to tau hyperphosphorylation, apoptosis, and cell death [21,

181]. The contribution of activated GSK3β and tau to cell death in the presence of model Hirano bodies was investigated. Exogenous expression of a constitutively active mutant of GSK3β (S9A) causes cell death similar to that of AICD, and that CT-GFP expression has no effect on this death (figure 3A, white bars). In contrast, exogenous expression of GSK3β (S9A) in the presence of 352 wt tau or 352PHP tau causes significant potentiation of cell death compared to GSK3β (S9A) alone (figure 3A, black bars ***$p < 0.001$, **$p < 0.01$). Interestingly, although 352 wt tau and 352PHP tau produce similar amounts of cell death under these conditions, 441 wt tau expression causes only an incremental increase in cell death when expressed with GSK3β S9A. Exogenous expression of GSK3β (S9A) with either wt tau isoforms or 352PHP tau in the presence of model Hirano bodies causes even further potentiation of cell death (figure 3A, check bars).

The effect of FTDP-17 tau expression on cell death induced by GSK3β (S9A) and model Hirano bodies was investigated. Exogenous expression of tau mutants R5H, P301L, and R406W in the presence of GSK3β (S9A) increased cell death compared to expression of GSK3β (S9A) alone (figure 3B, black bars). However, expression of G272V tau in the presence of GSK3β (S9A) resulted in only incremental increases in cell death compared to expression of GSK3β (S9A) alone. We also observed differences in the ability of model Hirano bodies to modulate cell death in the presence of FTDP-17 mutant tau and GSK3β (S9A). Model Hirano bodies enhanced cell death in the presence of GSK3β (S9A)+G272V tau and GSK3β (S9A)+P301L tau. (figure 3B, check bars ***$p < 0.001$). However, model Hirano bodies had no effect on cell death induced by GSK3β (S9A)+R5H tau or GSK3β (S9A)+R406W tau (figure 3B, check bars).

GSK3β (S9A) also enhanced cell death in the presence of K18 tau or K18ΔK280 tau compared to expression of GSK3β (S9A) alone (figure 3C, black bars ***$p < 0.001$). Model Hirano bodies did not have a statistically significant effect on this cell death although exogenous CT-GFP+GSK3β (S9A)+K18 tau resulted in a small increase in cell death compared to exogenous GSK3β (S9A)+K18 tau (figure 3C, check bars). We also measured cell death under conditions in which expression of GSK3β (S9A) did not induce significantly greater levels of cell death than GFP controls (figure 3D), showing that GSK3β (S9A)-induced cell death is dose dependent. Similar to previous results (figure 3A-C), exogenous expression of GSK3β (S9A)+441 wt tau or GSK3β (S9A)+P301L tau potentiated cell death compared to GSK3β (S9A) alone (figure 3D, black bars). In addition, the presence of model Hirano bodies further increased this cell death compared to expression of GSK3β (S9A)+441 wt tau (check bars, *$p < 0.05$) or GSK3β (S9A)+P301L tau (check bars ***$p < 0.001$). These results show that model Hirano bodies are not protective against cell death induced by GSK3β and tau, and depending on the mutation, may further increase cell death.

Discussion

Our results show that tau colocalizes with model Hirano bodies (figure 1B) consistent with previous studies [89, 107, 108]. However, model Hirano bodies only weakly colocalize with pseudohyperphosphorylated tau (352PHP). Model Hirano bodies mitigate cell death induced by AICD and AICD in the presence of wt tau isoforms, 352PHP, and tau mutants R5H, R406W, K18, and K18ΔK280 (figure 2A,B,D). In contrast, we show that model Hirano bodies potentiate cell death in the presence of G272V tau and P301L tau (figure 2C). Similarly, model Hirano bodies do not protect

from cell death in the presence of AICD+G272V tau or AICD+P301L tau (figure 3C). We also show that model Hirano bodies potentiate cell death induced by expression of activated GSK3β (S9A) and wt tau isoforms, 352 PHP, and tau mutants G272V and P301L (figure 3A,B). However, model Hirano bodies have no effect on cell death induced by GSK3β (S9A) and tau mutants R5H, R406W, K18, or K18ΔK280 (figure 3B,C).

Tau associates with Hirano bodies

Previous reports support the hypothesis that tau associates with Hirano bodies [89, 107, 108]. Moreover, multiple studies point to the role of tau as an actin binding protein [103, 106, 130, 132-134, 136, 137]. However, this idea is not without controversy. Studies of tau-actin interactions utilize a range of experimental approaches that yield differential results [182]. Nonetheless, we demonstrate that wt tau, as well as tau mutants known to cause FTDP-17 (R5H, G272V, P301L, and R406W) colocalize with model Hirano bodies in H4 cells (figure 1B). Further, tau fragments containing only the microtubule binding domain (K18 or K18ΔK280) were sufficient to associate with model Hirano bodies. This supports previous reports demonstrating that the microtubule binding domain of tau is capable of binding F-actin alone [134], and underscores the importance of this region in cytoskeletal interactions. Additionally, a tau mutant known to promote its self-aggregation (K18ΔK280) was sufficient to associate with model Hirano bodies. In contrast, a tau phosphomimic (352PHP) showed only weak colocalization with model Hirano bodies. This is supported by data showing that tau phosphorylation reduces its interaction with F-actin in vitro [129]. It is also well known that tau phosphorylation at multiple sites in vivo decreases its association with microtubules [183, 184]. The ability

of tau to bind actin may also be related to ability of tau to promote cell death in the presence of model Hirano bodies, and further investigation is needed to determine whether changes in the actin binding ability of tau correlates with changes in cell death.

Model Hirano bodies differentially modulate cell death in the presence of FTDP-17 tau

The ability of tau to promote cell death in the presence of model Hirano bodies and/or AICD correlates most strongly with the propensity of tau to self-aggregate. Multiple studies show that FTDP-17 tau mutants vary widely in their ability to form PHFs and NFTs in vitro, in cell culture, and in vivo (Table 2). Tau mutants P301L and K18ΔK280 show the greatest tendency to aggregate, and these mutants potentiated the highest levels of cell death in the presence of model Hirano bodies. Additionally, model Hirano bodies were not able to protect against these aggregation-prone tau mutants in the presence of AICD. In contrast, wt tau isoforms and tau mutants R406W and R5H did not cause cell death in the presence of model Hirano bodies, and model Hirano bodies were able to protect from cell death induced by these tau mutants in the presence of AICD. Although little is known about the R5H tau mutation, R406W tau does not robustly aggregate in vitro or in cell cultures (Table 1). In fact, R406W tau has been shown to be hypophosphorylated in resting cells compared to wt tau [144, 185], and hypophosphorylated when exposed to GSK3β in vitro [186]. Although the R5H and R406W mutations are on opposite sides of the tau molecule, residue 5 and 406 exist in close proximity upon the normal paperclip-like folding of tau [49, 187], and it is conceivable that the two mutations result in similar losses/gains of function. Thus, tau processing may be related to tau aggregation and cell death. The specific tau species responsible for potentiating cell death in our model is unknown, and more studies are

needed to determine whether further tau modification contributes to cell death. In support of a relationship between aggregation and cell death, some FTDP-17 patients exhibit more aggressive symptoms than AD patients, coincident with robust tau pathology at an earlier age of onset (for review, see [72]).

All tau mutants except for K18ΔK280 tau were incapable of inducing cell death alone consistent with previous reports [146, 147]. The ability of K18ΔK280 tau to cause cell death was attributed to the ability of this fragment to aggregate since mutation of hexapeptide motifs in tau essential for β-structure and aggregation reduced cell death and rescued neurodegeneration [73, 145]. Expression of P301L tau or G272V tau in the presence of model Hirano bodies significantly increased cell death. Model Hirano bodies have no effect on cell death initiated by expression of exogenous K18ΔK280 tau. However, because this mutant is capable of initiating cell death on its own, it likely does not need model Hirano bodies to further aid its aggregation. A possible mechanism for the interaction of model Hirano bodies and tau is that model Hirano bodies increase the local concentration of tau through a direct or indirect interaction. Tau mutants most prone to aggregation then initiate cell death. These results are consistent with studies showing that cell stress or neurodegeneration facilitates dramatic F-actin rearrangement in the presence of wt or mutant tau [103, 106].

Hirano bodies, tau, and AICD

Multiple reports suggest that AICD is likely important to the pathogenesis of AD [21-24]. However, this idea is controversial [188]. Transgenic mice expressing AICD and Fe65 exhibit classical AD-like deficits including increased hyperphosphorylated, insoluble tau and cognitive decline not caused by amyloid beta [21]. Conversely, Fe65

can bind Teashirt, forming a multi-protein complex to silence expression of caspase-4 [189].

Our data is consistent with the idea that tau significantly contributes to cell death in the presence of AICD. This complements previous results showing that expression of exogenous c31/APP/352PHP tau or AICD/352PHP tau results in a potentiation of cell death compared to cells expressing AICD or 352PHP alone [165]. In this study, co-expression of wt 352 or 441 tau and AICD produced a similar level of cell death to AICD alone. Cell death initiated by 441 tau and AICD was not statistically significant from cell death induced by AICD alone. Cell death induced by 352 wt tau and AICD was also modest relative to cell death caused by AICD alone. In contrast, expression of all tau mutants tested enhanced cell death in the presence of AICD (albeit at different levels) relative to expression of AICD or tau alone. These results support a model in which AICD potentiates cell death in the presence of tau through an unknown mechanism. Interestingly, model Hirano bodies reduced cell death initiated by K18ΔK280 tau and AICD, although this tau variant has the greatest propensity to aggregate (Table 1). This may be because K18ΔK280 tau is largely already processed into a pathological tau species, and does not need to undergo the same further processing compared to full-length tau mutants P301L or G272V. This further suggests that model Hirano bodies may promote early tau processing and/or aggregation depending on the tau species present.

Model Hirano bodies differentially potentiate cell death induced by GSK3β and tau.

Multiple studies suggest that a downstream effector of Aβ and AICD is the kinase GSK3β. Studies in neuronal cell cultures report upregulation of total levels of GSK3β as well as increased activation, and tau hyperphosphorylation [18, 21, 152]. Further, tau

hyperphosphorylation promotes tau aggregation in some models [49, 190, 191]. To test the hypothesis that model Hirano bodies protect against hyperphosphorylated tau, we utilized a constitutively active GSK3β mutant created by ablation of its inhibitory phosphorylation site (S9A). Expression of GSK3β alone induced moderate cell death, consistent with previous reports implicating a role for GSK3β in facilitating apoptosis [192, 193]. In all tau constructs tested except for R5H, R406W, K18, or K18ΔK280, expression of exogenous GSK3β and tau in the presence of model Hirano bodies strongly potentiated cell death compared to expression of GSK3β and tau. These results suggest that GSK3β induces formation of hyperphosphorylated tau species, that in the presence of model Hirano bodies could potentiate cell death by increasing the local concentration of tau. Interestingly, model Hirano bodies did not further enhance cell death in the presence of a tau mutant shown previously to be hypophosphorylated in the presence of GSK3β, or tau mutants that did not contain the majority of GSK3β phosphorylation sites (K18 and K18ΔK280). This may suggest a complementary role for phosphorylation and aggregation.

Notably, we observed similar results in the ability of model Hirano bodies to mitigate/enhance cell death in the presence of tau, tau+AICD, or tau+GSK3β. This may be because tau phosphorylation facilitates tau aggregation in our model. Given this, it is interesting that 352PHP tau produced low levels of cell death when expressed alone, and that model Hirano bodies were able to reduce cell death due to 352PHP tau and AICD. However, a mouse model of tauopathy created by overexpression of 352PHP tau failed to cause tau aggregation and neurodegeneration and resulted in a decrease in tau phosphorylation at certain sites compared to transgenic mice expressing wt tau [174].

Consistent with this, in vitro studies characterizing the 352PHP tau mutant show that these mutations collectively inhibit aggregation compared to wt tau [175]. Therefore, transient phosphorylation and dephosphorylation of tau may be critical to formation of a pathologically relevant tau species, and phosphorylation of specific residues likely affects modification at other sites. Indeed, GSK3β activity has been shown to differ between primed versus non-primed substrates [194]. These results support the hypothesis that model Hirano bodies enhance cell death in the presence of aggregation-prone tau, and reduce cell death in the presence of other tau variants.

Conclusions

In this study, we provide further evidence that Hirano bodies have a specific effect on the pathogenesis of neurodegenerative disease, with respect to tau and C-terminal fragments of APP. We suggest that Hirano bodies do not serve a general protective function by simply accumulating cytosolic proteins, but rather protect against cell death initiated by AICD and mitigate or enhance cell death dependent on the biochemical properties of tau. More work is needed to explore the function of Hirano bodies in vivo, and whether in vitro studies correlate or aid in interpretation of the results presented here. This data complements key discoveries made in transgenic animal models and neuronal cell culture reporting abnormal rearrangement of F-actin during cell stress or neurodegeneration, and suggest that Hirano bodies play a complex role in the pathogenesis of disease.

CHAPTER 3

CONCLUSIONS

In the current study, I have shown that tau associates with model Hirano bodies, an interaction that differentially affects cell death. Mutant tau proteins previously shown to have a greater propensity to aggregate compared to wt tau potentiate cell death in the presence of model Hirano bodies. Consistent with this, model Hirano bodies protect against cell death initiated by AICD and tau not prone to aggregation. Model Hirano bodies also potentiate cell death in the presence of GSK3β and tau, although not in the presence of tau fragments containing only the microtubule binding domain, or in the presence of tau mutants R406W and R5H. This is consistent with studies showing that hyperphosphorylation of regions adjacent to the microtubule binding domain of tau is important for aggregation and induction of cell death. However, more studies are needed to determine the mechanistic basis of cell death in our model.

Future directions: GSK3β

GSK3β was investigated due to previously published data implicating it as a target of AICD mediated signaling [18, 21]. In neuronal cell culture models, total GSK3β levels were increased and over-activated upon expression of exogenous AICD [18]. This resulted in abnormal tau phosphorylation and cell death. However, more studies are needed to determine whether this is occurring in our model. These studies could utilize antibodies against total GSK3β and activated GSK3β (phosphorylated Y216) and western blotting to determine if total or activated levels of GSK3β are modulated by AICD in the

presence or absence of model Hirano bodies. Further, a mutant form of GSK3β (K85A) results in ablation of the ATP binding site and is known to have a dominant negative effect [195]. Inhibitors are also commercially available, although the specificity of many inhibitors is debated due to high sequence and structural homology to other kinases [180]. GSK3β (K85A) could be expressed in the presence of AICD and AICD+tau to determine whether suppression of GSK3β mitigates cell death. Localization patterns of endogenous as well as exogenous epitope tagged GSK3β could also be performed to determine if GSK3β colocalizes with model Hirano bodies. If so, this may provide a mechanistic basis for further tau phosphorylation and increases in cell death, or conversely, decreased ability of GSK3β to modulate other processes contributing to cell death. However, I have found that model Hirano bodies have no affect on cell death, albeit at low levels, initiated by GSK3β. Although GSK3β is one particular downstream target of AICD, a growing number of AICD interacting partners and gene targets may also contribute to cell death (for review, see [150]). Previous data from our laboratory shows that AICD-dependent cell death involves, at least in part, tat-interacting protein 60 kDa (Tip60), p53, and caspases [165]. Interestingly, another study identified α2-actin, tropomyosin, and transgelin as gene targets of AICD using a microarray approach in primary cortical neurons, establishing a link between actin dynamics and AICD signaling [12].

Future directions: tau aggregation

I also found marked differences in the ability of tau mutants to promote cell death in the presence of model Hirano bodies, and a similar trend in the presence of AICD and model Hirano bodies. All mutants tested potentiated cell death in the presence of AICD to a greater extent than wt 352 or 441 tau. However, model Hirano bodies did not reduce

cell death potentiated by G272V tau+AICD or P301L tau+AICD. In contrast, model Hirano bodies protected against cell death potentiated by R5H tau+AICD or R406W+AICD. This trend may be related to the biochemical properties of tau. G272V, P301L, 441ΔK280, and K18ΔK280 have been shown to readily aggregate in vitro in the presence of polyanionic cofactors [74-76]. Further, 441ΔK280 tau has been shown to aggregate in the absence of cofactors in vitro [74], and form electron dense aggregates in stably transfected CHO cells [196], properties not shown for other tau mutants. Truncation of 441ΔK280 to K18ΔK280 further aids aggregation and causes significant levels of cell death in neuronal cell cultures and AD-like deficits in transgenic mice [145-147]. K18ΔK280 also caused significant cell death in my experiments, and model Hirano bodies failed to reduce this death. These data are consistent with the hypothesis that aggregation contributes significantly to cell death observed upon expression of G272V tau+CT-GFP or P301L tau+CT-GFP. However, further experiments are needed to characterize whether this process is occurring in our model. Structural studies aimed at determining the region of tau responsible for aggregation show that the presence of either 1 (in 3R tau) or 2 (in 4R tau) hexapeptide motifs are essential for formation of β structure and aggregation [73]. Ablation of these motifs by mutating specific isoleucine residues to proline disrupts the potential for β structure and aggregation in tau. Interestingly, in cell culture and transgenic mouse models of tau aggregation, disruption of hexapeptide motifs by β-breaking mutations in K18ΔK280 tau rescue tau aggregation and neurodegeneration [145, 147]. In our model, if tau aggregation is in fact contributing to cell death, I would expect that introduction of these point mutations would at least partially rescue cell death. Other assays to measure tau aggregation could include thioflavinS fluorescence assays

that measure the total amyloidogenic protein present in cell lysates. Alternatively, separation of soluble/insoluble fractions and western blotting could reveal changes in insoluble tau levels. I have shown that a large fraction of exogenously expressed tau is colocalized with model Hirano bodies in most mutants tested in this study. Additionally, I hypothesized that model Hirano bodies may contribute to cell death observed in the presence of P301L tau and G272V tau by increasing the local contribution of tau near the model Hirano body. Thus, it may be useful to utilize transmission electron microscopy (TEM) to detect localization of fibrillar structures relative to model Hirano bodies. Further, Hirano bodies exhibit a characteristic ultrastructure that may be modified by tau expression. Recent studies in our laboratory (Connor Sweetnam, unpublished data) show that tau does not induce Hirano body formation or result in significantly greater numbers of Hirano bodies contrary to one previous study [197]. On the contrary, expression of 441 wt tau, 352 wt tau, or P301L tau in the presence of CT-GFP results in decreased numbers of model Hirano bodies in H4 cells compared to expression of CT-GFP alone.

Future directions: post-translation modification of tau

Multiple post-translational modifications could also contribute to the differential cell death observed between tau mutants. Among the modifications thought to facilitate tau aggregation are phosphorylation at specific residues [191, 198], acetylation [199, 200], and truncation by various proteases [201]. It is possible, for example that G272V tau and P301L tau are differentially phosphorylated at specific sites compared to wt tau, R5H tau, R406W tau, or 352PHP tau, and it is known that phosphorylation at different residues results in different functional outcomes. For example, GSK3β preferentially targets substrates that have been prephosphorylated by other priming kinases [202].

Indeed, GSK3β activity has been shown to differ between primed versus non-primed substrates [194]. This may account for differences in cell death seen between 352PHP tau, G272V tau, and P301L tau in the presence of AICD and model Hirano bodies. In addition, a mouse model of tauopathy created by overexpression of 352PHP tau failed to cause tau aggregation and neurodegeneration and resulted in a decrease in tau phosphorylation at certain sites compared to transgenic mice expressing wt tau [174]. Consistent with this, in vitro studies characterizing the 352PHP tau mutant show that these mutations collectively inhibit aggregation compared to wt tau [175]. Therefore, transient phosphorylation and dephosphorylation of tau may be critical to formation of a pathologically relevant tau species, and phosphorylation of specific residues likely affects phosphorylation and modification at other sites. Given this, it may be useful to measure whether tau phosphorylation differs among experimental conditions used in this study, and whether this correlates with cell death. This can be accomplished by western blotting using antibodies that recognize particular phosphorylated tau epitopes. These same antibodies can also be used to determine the subcellular localization of tau during different conditions. In this study, tau localization was measured only in the presence of CT-GFP. However, more colocalization studies are needed to investigate changes in tau localization in the presence of AICD and model Hirano bodies. Upon transfection of AICD and tau, differential tau modification could affect its localization with Hirano bodies. If AICD expression results in increased tau phosphorylation, for example, the colocalization between tau and model Hirano bodies could be abolished. In support of this, I report that 352PHP tau does not show strong colocalization with model Hirano bodies. However, other post-translational modifications may contribute to increased cell

death in the presence of AICD. In an unbiased approach, tau expressed in the presence of AICD could be purified and analyzed by mass spectrometry.

Future directions: tau-actin binding studies

Differential tau-actin binding likely has important consequences in vivo, because tau is predominantly localized to the distal axon in mature neurons [158], an interaction that is dependent on intact F-actin [136, 137]. Hyperphosphorylation or F-actin depolymerization destroys axonal retention of tau, resulting in its accumulation in the somatodendritic compartment [136, 203]. In early AD, this mediates destruction of actin-rich dendritic spines and synaptic dysfunction [42, 48, 203]. I have shown that wt tau as well as tau mutants colocalize with model Hirano bodies. However, this does not prove a direct interaction and more studies are needed to determine whether this interaction is mediated by other factors. No studies to date have attempted to determine the ability of tau mutants to bind actin relative to wt tau. Certain FTDP-17 tau mutants as well as 352PHP tau have been shown to have decreased ability to bind microtubules [140, 204], and the same region of tau responsible for microtubule binding is also important for actin binding [134]. Therefore, tau mutations and phosphorylation may affect actin binding. To this end, I have generated all previously tested tau mutants in bacterial expression plasmids (pET 29b, Novagen) for purification. Future experiments in our laboratory will be focused on determining the actin binding capability of FTDP-17 tau mutants and 352PHP tau compared to wt tau using a surface plasmon resonance technique (Biacore) [205, 206].

Future directions: In vivo models

More studies are needed in relevant cell culture and in vivo models to adequately determine the contribution of tau to cell death in the presence of model Hirano bodies and AICD. Specifically, it would be useful to determine if endogenous tau colocalizes with model Hirano bodies in primary neuronal cultures, or in human neuronal iPS cells that express endogenous tau. Antibodies to different phosphorylated states of tau as well as different isoforms could be used with and without AICD to further characterize the interactions between tau and Hirano bodies. Studies show that tau colocalizes with Hirano bodies in post-mortem human brain tissue, but this is not a widely accepted phenomenon [154]. In addition, epitopes used to generate antibodies in these studies are somewhat unknown, and may include other MAPs. Therefore, a more exhaustive study of tau colocalization with Hirano bodies in human brain material is needed and would reveal the specific isoforms and modifications that facilitate this interaction. Lastly, various transgenic mouse models of tauopathy are available that express mutant forms of tau that recapitulate key aspects of AD and FTDP-17 [142, 207, 208]. Genetic crosses of our Hirano body model mice with these tauopathy mice would help to characterize the putative interaction between tau and Hirano bodies, and their relationship to the formation of tau pathology, electrophysiology, and behavior.

In conclusion, the data presented here provides the first detailed study of the association between pathological tau mutants and Hirano bodies, and is the first to demonstrate the potential consequences of this interaction as it relates to cell death. The contribution of AICD to AD and FTLD-tau is poorly understood and may play an important role in human disease as well as in various mouse models of AD that use

FTDP-17 tau mutants to recapitulate tau pathology. These results extend previous reports affirming a specific role for Hirano bodies in aging and disease states and provide further evidence that the presence of Hirano bodies may have an important contribution to the pathogenesis of neurodegenerative disease.

References

1. Zheng H, Koo EH: **Biology and pathophysiology of the amyloid precursor protein**. *Mol Neurodegener* 2011, **6**(1):27.
2. Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS, Wisniewski HM: **Microtubule-associated protein tau. A component of Alzheimer paired helical filaments**. *The Journal of Biological Chemistry* 1986, **261**(13):6084-6089.
3. Galloway PG, Perry G, Gambetti P: **Hirano body filaments contain actin and actin-associated proteins**. *J Neuropathol Exp Neurol* 1987, **46**(2):185-199.
4. Spires-Jones TL, Kopeikina KJ, Koffie RM, de Calignon A, Hyman BT: **Are tangles as toxic as they look?** *J Mol Neurosci* 2011, **45**(3):438-444.
5. Karran E, Mercken M, De Strooper B: **The amyloid cascade hypothesis for Alzheimer's disease: an appraisal for the development of therapeutics**. *Nat Rev Drug Discov* 2011, **10**(9):698-712.
6. Huang Y, Mucke L: **Alzheimer mechanisms and therapeutic strategies**. *Cell* 2012, **148**(6):1204-1222.
7. De Strooper B, Saftig P, Craessaerts K, Vanderstichele H, Guhde G, Annaert W, Von Figura K, Van Leuven F: **Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein**. *Nature* 1998, **391**(6665):387-390.
8. Yan R, Bienkowski MJ, Shuck ME, Miao H, Tory MC, Pauley AM, Brashier JR, Stratman NC, Mathews WR, Buhl AE, Carter DB, Tomasselli AG, Parodi LA,

Heinrikson RL, Gurney ME: **Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity**. *Nature* 1999, **402**(6761):533-537.

9. Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, Davis D, Doan M, Dovey HF, Frigon N, Hong J, Jacobson-Croak K, Jewett N, Keim P, Knops J, Lieberburg I, Power M, Tan H, Tatsuno G, Tung J, Schenk D, Seubert P, Suomensaari SM, Wang S, Walker D, Zhao J, McConlogue L, John V: **Purification and cloning of amyloid precursor protein beta-secretase from human brain**. *Nature* 1999, **402**(6761):537-540.

10. Zhao M, Su J, Head E, Cotman CW: **Accumulation of caspase cleaved amyloid precursor protein represents an early neurodegenerative event in aging and in Alzheimer's disease**. *Neurobiol Dis* 2003, **14**(3):391-403.

11. Lu DC, Rabizadeh S, Chandra S, Shayya RF, Ellerby LM, Ye X, Salvesen GS, Koo EH, Bredesen DE: **A second cytotoxic proteolytic peptide derived from amyloid B-protein precursor**. *Nature Medicine* 2000, **6**:397-404.

12. Müller T, Concannon CG, Ward MW, Walsh CM, Tirniceriu AL, Tribl F, Kogel D, Prehn JHM, Egensperger R: **Modulation of gene expression and cytoskeletal dynamics of the amyloid precursor protein intracellular domain (AICD)**. *Mol Biol Cell* 2007, **18**:201-210.

13. Ha S, Furukawa R, Fechheimer M: **Association of AICD and Fe65 with Hirano bodies reduces transcriptional activation and initiation of apoptosis**. *Neurobiology of aging* 2011, **32**(12):2287-2298.

14. Ward MW, Concannon CG, Whyte J, Walsh CM, Corley B, Prehn JH: **The amyloid precursor protein intracellular domain(AICD) disrupts actin**

dynamics and mitochondrial bioenergetics. *Journal of neurochemistry* 2010, **113**(1):275-284.

15. Shaked GM, Kummer MP, Lu DC, Galvan V, Bredesen DE, Koo EH: **Abeta induces cell death by direct interaction wiwth its cognate extracellular domain on app (app597-624)**. *FASEB J* 2006, **20**:1254-1256.

16. Cao X, Südhof TC: **A transcriptionally active complex of APP with Fe65 and histone acetyltransferase Tip60**. *Science* 2001, **293**:115-120.

17. Cao X, Südhof TC: **Dissection of amyloid-b precursor protein-dependent transcriptional transactivation**. *J Biol Chem* 2004, **279**:24601-14611.

18. Kim H-S, Kim E-M, Lee J-P, Park CH, Kim S, Seo J-H, Chang K-A, Yu E, Jeong S-J, Chong YH, Suh AY-H: **C-terminal fragments of amyloid precursor protein exert neurotoxicity by inducing glycogen synthase kinase-3b expression**. *FASEB J* 2003, **17**:1951-1954.

19. Kimberly WT, Zheng JB, Guenette SY, Selkoe DJ: **The intracellular domain of the beta-amyloid precursor protein is stabilized by Fe65 and translocates to the nucleus in a notch-like manner**. *J Biol Chem* 2001, **276**:40288-40292.

20. Zambrano N, Minopoli G, de Candia P, Russo T: **The Fe65 adaptor protein interacts through its PID1 domain with the transcription factor CP2/FSF/LBP1**. *J Biol Chem* 1998, **273**:20128-20133.

21. Ghosal K, Vogt DL, Liang M, Shen Y, Lamb BT, Pimplikar SW: **Alzheimer's disease-like pathological features in transgenic mice expressing the APP intracellular domain**. *Proc Natl Acad Sci U S A* 2009, **106**:18367-18372.

22. Vogt DL, Thomas D, Galvan V, Bredesen DE, Lamb BT, Pimplikar SW: **Abnormal neuronal networks and seizure susceptibility in mice overexpressing the APP intracellular domain**. *Neurobiology of aging* 2011, **32**(9):1725-1729.

23. Ghosal K, Stathopoulos A, Pimplikar SW: **APP intracellular domain impairs adult neurogenesis in transgenic mice by inducing neuroinflammation**. *PLoS ONE* 2010, **5**(7):e11866.

24. Ryan KA, Pimplikar SW: **Activation of GSK-3 and phosphorylation of CRMP2 in transgenic mice expressing APP intracellular domain**. *The Journal of Cell Biology* 2005, **171**(2):327-335.

25. Delatour B, Mercken L, Hachimi KH, Colle MA, Padier L, Duyckaerts C: **FE65 in Alzheimer's disease: neuronal distribution and association with neurofibrillary tangles**. *Am J Clin Pathol* 2001, **158**:1585-1591.

26. Barbato C, Canu N, Zambrano N, Serafino A, Minopoli G, Ciotti MT, Amadoro G, Russo T, Calissano P: **Interaction of Tau with Fe65 links tau to APP**. *Neurobiol Dis* 2005, **18**(2):399-408.

27. Ermekova KS, Zambrano N, Linn H, Minopoli G, Gertler F, Russo T, Sudol M: **The WW domain of neural protein FE65 interacts with proline-rich motifs in mena, the mammalian homolog of Drosophila enabled**. *J Biol Chem* 1997, **272**:32869-32877.

28. Galvan V, Gorostiza OF, Banwait S, Ataie M, Logvinova AV, Sitaraman S, Carlson E, Sagi SA, Chevallier N, Jin K, Greenberg DA, Bredesen DE: **Reversal of Alzheimer's-like pathology and behavior in human APP transgenic mice**

by mutation of Asp664. *Proceedings of the National Academy of Sciences of the United States of America* 2006, **103**(18):7130-7135.

29. Galvan V, Zhang J, Gorostiza OF, Banwait S, Huang W, Ataie M, Tang H, Bredesen DE: **Long-term prevention of Alzheimer's disease-like behavioral deficits in PDAPP mice carrying a mutation in Asp664**. *Behav Brain Res* 2008, **191**(2):246-255.

30. Saganich MJ, Schroeder BE, Galvan V, Bredesen DE, Koo EH, Heinemann SF: **Deficits in synaptic transmission and learning in amyloid precursor protein (APP) transgenic mice require c-terminal cleavage of APP**. *J Neurosci* 2006, **26**:13428-13436.

31. Drechsel DN, Hyman AA, Cobb MH, Kirschner MW: **Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau**. *Molecular Biology of the Cell* 1992, **3**(10):1141-1154.

32. Weingarten MD, Lockwood AH, Hwo SY, Kirschner MW: **A protein factor essential for microtubule assembly**. *Proceedings of the National Academy of Sciences of the United States of America* 1975, **72**(5):1858-1862.

33. Dixit R, Ross JL, Goldman YE, Holzbaur EL: **Differential regulation of dynein and kinesin motor proteins by tau**. *Science* 2008, **319**(5866):1086-1089.

34. Ebneth A, Godemann R, Stamer K, Illenberger S, Trinczek B, Mandelkow E: **Overexpression of tau protein inhibits kinesin-dependent trafficking of vesicles, mitochondria, and endoplasmic reticulum: implications for Alzheimer's disease**. *The Journal of Cell Biology* 1998, **143**(3):777-794.

35. Zmuda JF, Rivas RJ: **Actin disruption alters the localization of tau in the growth cones of cerebellar granule neurons**. *Journal of Cell Science* 2000, **113 (Pt 15)**:2797-2809.

36. Takei Y, Teng J, Harada A, Hirokawa N: **Defects in axonal elongation and neuronal migration in mice with disrupted tau and map1b genes**. *The Journal of Cell Biology* 2000, **150**(5):989-1000.

37. Harada A, Oguchi K, Okabe S, Kuno J, Terada S, Ohshima T, Sato-Yoshitake R, Takei Y, Noda T, Hirokawa N: **Altered microtubule organization in small-calibre axons of mice lacking tau protein**. *Nature* 1994, **369**(6480):488-491.

38. Dawson HN, Ferreira A, Eyster MV, Ghoshal N, Binder LI, Vitek MP: **Inhibition of neuronal maturation in primary hippocampal neurons from tau deficient mice**. *Journal of Cell Science* 2001, **114**(Pt 6):1179-1187.

39. Ikegami S, Harada A, Hirokawa N: **Muscle weakness, hyperactivity, and impairment in fear conditioning in tau-deficient mice**. *Neuroscience letters* 2000, **279**(3):129-132.

40. Lei P, Ayton S, Finkelstein DI, Spoerri L, Ciccotosto GD, Wright DK, Wong BX, Adlard PA, Cherny RA, Lam LQ, Roberts BR, Volitakis I, Egan GF, McLean CA, Cappai R, Duce JA, Bush AI: **Tau deficiency induces parkinsonism with dementia by impairing APP-mediated iron export**. *Nature Medicine* 2012, **18**(2):291-295.

41. Sultan A, Nesslany F, Violet M, Begard S, Loyens A, Talahari S, Mansuroglu Z, Marzin D, Sergeant N, Humez S, Colin M, Bonnefoy E, Buee L, Galas MC:

Nuclear tau, a key player in neuronal DNA protection. *The Journal of Biological Chemistry* 2011, **286**(6):4566-4575.

42. Ittner LM, Ke YD, Delerue F, Bi M, Gladbach A, van Eersel J, Wolfing H, Chieng BC, Christie MJ, Napier IA, Eckert A, Staufenbiel M, Hardeman E, Gotz J: **Dendritic function of tau mediates amyloid-beta toxicity in Alzheimer's disease mouse models**. *Cell* 2010, **142**(3):387-397.

43. Martin L, Latypova X, Terro F: **Post-translational modifications of tau protein: implications for Alzheimer's disease**. *Neurochem Int* 2011, **58**(4):458-471.

44. Bancher C, Grundke-Iqbal I, Iqbal K, Fried VA, Smith HT, Wisniewski HM: **Abnormal phosphorylation of tau precedes ubiquitination in neurofibrillary pathology of Alzheimer disease**. *Brain Res* 1991, **539**(1):11-18.

45. Wood JG, Mirra SS, Pollock NJ, Binder LI: **Neurofibrillary tangles of Alzheimer disease share antigenic determinants with the axonal microtubule-associated protein tau (tau)**. *Proceedings of the National Academy of Sciences of the United States of America* 1986, **83**(11):4040-4043.

46. Nukina N, Ihara Y: **One of the antigenic determinants of paired helical filaments is related to tau protein**. *J Biochem* 1986, **99**(5):1541-1544.

47. Tackenberg C, Brandt R: **Divergent pathways mediate spine alterations and cell death induced by amyloid-beta, wild-type tau, and R406W tau**. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2009, **29**(46):14439-14450.

48. Hoover BR, Reed MN, Su J, Penrod RD, Kotilinek LA, Grant MK, Pitstick R, Carlson GA, Lanier LM, Yuan LL, Ashe KH, Liao D: **Tau mislocalization to**

dendritic spines mediates synaptic dysfunction independently of neurodegeneration. *Neuron* 2010, **68**(6):1067-1081.

49. Jeganathan S, Hascher A, Chinnathambi S, Biernat J, Mandelkow EM, Mandelkow E: **Proline-directed pseudo-phosphorylation at AT8 and PHF1 epitopes induces a compaction of the paperclip folding of Tau and generates a pathological (MC-1) conformation**. *The Journal of Biological Chemistry* 2008, **283**(46):32066-32076.

50. SantaCruz K, Lewis J, Spires T, Paulson J, Kotilinek L, Ingellsson M, Guimaraes A, DeTure M, Ramsden M, McGowan E, Forster C, Yue M, Orne J, Janus C, Mariash A, Kiskowski M, Hyman B, Hutton M, Ashe KH: **Tau Suppression in a Neurodegenerative Mouse Model Improves Memory Function**. *Science* 2005, **309**:476-481.

51. Roberson ED, Scearce-Levie K, Palop JJ, Yan F, Cheng IH, Wu T, Gerstein H, Yu G-Q, Mucke L: **Reducing endogenous tau ameliorates amyloid b-induced deficits in an Alzheimer's disease mouse model**. *Science* 2007, **316**:750-754.

52. Rapoport M, Dawson HN, Binder LI, Vitek MP, Ferreira A: **Tau is essential to beta -amyloid-induced neurotoxicity**. *Proceedings of the National Academy of Sciences of the United States of America* 2002, **99**(9):6364-6369.

53. Jin M, Shepardson N, Yang T, Chen G, Walsh D, Selkoe DJ: **Soluble amyloid beta-protein dimers isolated from Alzheimer cortex directly induce Tau hyperphosphorylation and neuritic degeneration**. *Proceedings of the National Academy of Sciences of the United States of America* 2011, **108**(14):5819-5824.

54. Hardy J, Selkoe DJ: **The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics**. *Science* 2002, **297**:353-356.

55. Dodart JC, Bales KR, Gannon KS, Greene SJ, DeMattos RB, Mathis C, DeLong CA, Wu S, Wu X, Holtzman DM, Paul SM: **Immunization reverses memory deficits without reducing brain Abeta burden in Alzheimer's disease model**. *Nature Neuroscience* 2002, **5**(5):452-457.

56. Ferreira A, Lu Q, Orecchio L, Kosik KS: **Selective phosphorylation of adult tau isoforms in mature hippocampal neurons exposed to fibrillar A beta**. *Mol Cell Neurosci* 1997, **9**(3):220-234.

57. Greenberg SM, Koo EH, Selkoe DJ, Qiu WQ, Kosik KS: **Secreted beta-amyloid precursor protein stimulates mitogen-activated protein kinase and enhances tau phosphorylation**. *Proceedings of the National Academy of Sciences of the United States of America* 1994, **91**(15):7104-7108.

58. Morris M, Maeda S, Vossel K, Mucke L: **The many faces of tau**. *Neuron* 2011, **70**(3):410-426.

59. Mackenzie IR, Neumann M, Bigio EH, Cairns NJ, Alafuzoff I, Kril J, Kovacs GG, Ghetti B, Halliday G, Holm IE, Ince PG, Kamphorst W, Revesz T, Rozemuller AJ, Kumar-Singh S, Akiyama H, Baborie A, Spina S, Dickson DW, Trojanowski JQ, Mann DM: **Nomenclature for neuropathologic subtypes of frontotemporal lobar degeneration: consensus recommendations**. *Acta neuropathologica* 2009, **117**(1):15-18.

60. Lee VM, Goedert M, Trojanowski JQ: **Neurodegenerative tauopathies**. *Ann Rev Neurosci* 2001, **24**:1121-1159.

61. Hauw JJ, Verny M, Delaere P, Cervera P, He Y, Duyckaerts C: **Constant neurofibrillary changes in the neocortex in progressive supranuclear palsy. Basic differences with Alzheimer's disease and aging**. *Neuroscience letters* 1990, **119**(2):182-186.

62. Yamada T, McGeer PL, McGeer EG: **Appearance of paired nucleated, Tau-positive glia in patients with progressive supranuclear palsy brain tissue**. *Neuroscience letters* 1992, **135**(1):99-102.

63. Komori T: **Tau-positive glial inclusions in progressive supranuclear palsy, corticobasal degeneration and Pick's disease**. *Brain Pathol* 1999, **9**(4):663-679.

64. Flament S, Delacourte A, Verny M, Hauw JJ, Javoy-Agid F: **Abnormal Tau proteins in progressive supranuclear palsy. Similarities and differences with the neurofibrillary degeneration of the Alzheimer type**. *Acta neuropathologica* 1991, **81**(6):591-596.

65. Vermersch P, Robitaille Y, Bernier L, Wattez A, Gauvreau D, Delacourte A: **Biochemical mapping of neurofibrillary degeneration in a case of progressive supranuclear palsy: evidence for general cortical involvement**. *Acta neuropathologica* 1994, **87**(6):572-577.

66. Feany MB, Dickson DW: **Widespread cytoskeletal pathology characterizes corticobasal degeneration**. *Am J Pathol* 1995, **146**(6):1388-1396.

67. Feany MB, Mattiace LA, Dickson DW: **Neuropathologic overlap of progressive supranuclear palsy, Pick's disease and corticobasal degeneration**. *Journal of neuropathology and experimental neurology* 1996, **55**(1):53-67.

68. Sergeant N, Wattez A, Delacourte A: **Neurofibrillary degeneration in progressive supranuclear palsy and corticobasal degeneration: tau pathologies with exclusively "exon 10" isoforms**. *Journal of neurochemistry* 1999, **72**(3):1243-1249.

69. Goedert M, Jakes R: **Mutations causing neurodegenerative tauopathies**. *Biochim Biophys Acta* 2005, **1739**(2-3):240-250.

70. Foster NL, Wilhelmsen K, Sima AA, Jones MZ, D'Amato CJ, Gilman S: **Frontotemporal dementia and parkinsonism linked to chromosome 17: a consensus conference. Conference Participants**. *Ann Neurol* 1997, **41**(6):706-715.

71. Wilhelmsen KC, Lynch T, Pavlou E, Higgins M, Nygaard TG: **Localization of disinhibition-dementia-parkinsonism-amyotrophy complex to 17q21-22**. *Am J Hum Genet* 1994, **55**(6):1159-1165.

72. Wszolek ZK, Tsuboi Y, Ghetti B, Pickering-Brown S, Baba Y, Cheshire WP: **Frontotemporal dementia and parkinsonism linked to chromosome 17 (FTDP-17)**. *Orphanet J Rare Dis* 2006, **1**:30.

73. von Bergen M, Friedhoff P, Biernat J, Heberle J, Mandelkow EM, Mandelkow E: **Assembly of tau protein into Alzheimer paired helical filaments depends on a local sequence motif ((306)VQIVYK(311)) forming beta structure**. *Proceedings of the National Academy of Sciences of the United States of America* 2000, **97**(10):5129-5134.

74. von Bergen M, Barghorn S, Li L, Marx A, Biernat J, Mandelkow EM, Mandelkow E: **Mutations of tau protein in frontotemporal dementia promote**

aggregation of paired helical filaments by enhancing local beta-structure. *The Journal of Biological Chemistry* 2001, **276**(51):48165-48174.

75. Barghorn S, Zheng-Fischhofer Q, Ackmann M, Biernat J, von Bergen M, Mandelkow EM, Mandelkow E: **Structure, microtubule interactions, and paired helical filament aggregation by tau mutants of frontotemporal dementias**. *Biochemistry* 2000, **39**(38):11714-11721.

76. Goedert M, Jakes R, Crowther RA: **Effects of frontotemporal dementia FTDP-17 mutations on heparin-induced assembly of tau filaments**. *FEBS Letters* 1999, **450**(3):306-311.

77. D'Souza I, Schellenberg GD: **Regulation of tau isoform expression and dementia**. *Biochimica et Biophysica Acta* 2005, **1739**(2-3):104-115.

78. Lee EB, Lee VM, Trojanowski JQ: **Gains or losses: molecular mechanisms of TDP43-mediated neurodegeneration**. *Nat Rev Neurosci* 2012, **13**(1):38-50.

79. Mackenzie IR, Rademakers R, Neumann M: **TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia**. *Lancet Neurol* 2010, **9**(10):995-1007.

80. Geser F, Lee VM, Trojanowski JQ: **Amyotrophic lateral sclerosis and frontotemporal lobar degeneration: a spectrum of TDP-43 proteinopathies**. *Neuropathology* 2010, **30**(2):103-112.

81. Momeni P, Pittman A, Lashley T, Vandrovcova J, Malzer E, Luk C, Hulette C, Lees A, Revesz T, Hardy J, de Silva R: **Clinical and pathological features of an Alzheimer's disease patient with the MAPT Delta K280 mutation**. *Neurobiology of aging* 2009, **30**(3):388-393.

82. Hayashi S, Toyoshima Y, Hasegawa M, Umeda Y, Wakabayashi K, Tokiguchi S, Iwatsubo T, Takahashi H: **Late-onset frontotemporal dementia with a novel exon 1 (Arg5His) tau gene mutation**. *Ann Neurol* 2002, **51**(4):525-530.

83. Mirra SS, Murrell JR, Gearing M, Spillantini MG, Goedert M, Crowther RA, Levey AI, Jones R, Green J, Shoffner JM, Wainer BH, Schmidt ML, Trojanowski JQ, Ghetti B: **Tau pathology in a family with dementia and a P301L mutation in tau**. *Journal of neuropathology and experimental neurology* 1999, **58**(4):335-345.

84. Miyasaka T, Morishima-Kawashima M, Ravid R, Kamphorst W, Nagashima K, Ihara Y: **Selective deposition of mutant tau in the FTDP-17 brain affected by the P301L mutation**. *Journal of neuropathology and experimental neurology* 2001, **60**(9):872-884.

85. van Swieten JC, Stevens M, Rosso SM, Rizzu P, Joosse M, de Koning I, Kamphorst W, Ravid R, Spillantini MG, Niermeijer, Heutink P: **Phenotypic variation in hereditary frontotemporal dementia with tau mutations**. *Ann Neurol* 1999, **46**(4):617-626.

86. Hirano A, Dembitzer HM, Kurland LT, Zimmerman HM: **The Fine Structure of Some Intraganlionic Alterations**. *J Neuropathol Expt Neurol* 1968, **27**(2):167-182.

87. Schochet SS, Jr., Lampert PW, Lindenberg R: **Fine structure of the Pick and Hirano bodies in a case of Pick's disease**. *Acta Neuropathol (Berl)* 1968, **11**(4):330-337.

88. Goldman JE: **The association of actin with Hirano bodies**. *J Neuropathol Exp Neurol* 1983, **42**(2):146-152.

89. Davis RC, Furukawa R, Fechheimer M: **A cell culture model for investigation of Hirano bodies**. *Acta Neuropathol* 2008, **115**(2):205-217.

90. Ogata J, Budzilovich GN, Cravioto H: **A study of rod-like structures (Hirano bodies) in 240 normal and pathological brains**. *Acta Neuropathol* 1972, **21**(1):61-67.

91. Gibson PH, Tomlinson BE: **Numbers of Hirano bodies in the hippocampus of normal and demented people with Alzheimer's disease**. *J Neurol Sci* 1977, **33**(1-2):199-206.

92. Gibson PH: **Light and electron microscopic observations on the relationship between Hirano bodies, neuron and glial perikarya in the human hippocampus**. *Acta Neuropathol (Berl)* 1978, **42**(3):165-171.

93. Mori H, Tomonaga M, Baba N, Kanaya K: **The structure analysis of Hirano bodies by digital processing on electron micrographs**. *Acta Neuropathol* 1986, **71**(1-2):32-37.

94. Martinez-Saez E, Gelpi E, Rey M, Ferrer I, Ribalta T, Botta-Orfila T, Nos C, Yague J, Sanchez-Valle R: **Hirano body - rich subtypes of Creutzfeldt-Jakob disease**. *Neuropathology and applied neurobiology* 2011.

95. Cartier L, Galvez S, Gajdusek DC: **Familial clustering of the ataxic form of Creutzfeldt-Jakob disease with Hirano bodies**. *Journal of neurology, neurosurgery, and psychiatry* 1985, **48**(3):234-238.

96. Guiroy DC, Liberski PP, Williams ES, Gajdusek DC: **Electron microscopic findings in brain of Rocky Mountain elk with chronic wasting disease**. *Folia Neuropathol* 1994, **32**(3):171-173.

97. Baloyannis SJ, Manolidis SL, Manolidis LS: **Synaptic alterations in acoustic cortex in Creutzfeldt-Jacob disease**. *Acta Otolaryngol* 1995, **115**(2):202-205.

98. Sima AA, Hinton D: **Hirano-bodies in the distal symmetric polyneuropathy of the spontaneously diabetic BB-Wistar rat**. *Acta Neurol Scand* 1983, **68**(2):107-112.

99. Laas R, Hagel C: **Hirano bodies and chronic alcoholism**. *Neuropathol Appl Neurobiol* 1994, **20**(1):12-21.

100. Wegiel J, K. C. Wang KC, Imaki H, Rubenstein R, Wronska A, Osuchowski M, Lipinski WJ, Walker LC, LeVine H: **The role of microglial cells and astrocytes in fibrillar plaque evolution in transgenic APP(SW) mice.** *Neurobiol Aging* 2001, **22**:49-61.

101. Masliah E, Sisk A, Mallory M, Mucke L, Schenk D, Games D: **Comparison of neurodegenerative pathology in transgenic mice overexpressing V717F beta-amyloid precursor protein and Alzheimer's disease**. *J Neurosci* 1996, **16**:5795-5811.

102. Boutajangout A, Authelet M, Blanchard V, Touchet N, Tremp G, Pradier L, Brion JP: **Characterisation of cytoskeletal abnormalities in mice transgenic for wild-type human tau and familial Alzheimer's disease mutants of APP and presenilin-1**. *Neurobiol Dis* 2004, **15**:47-60.

103. Fulga TA, Elson-Schwab I, Khurana V, Steinhib ML, Spires TL, T. HB, Feany MB: **Abnormal bundling and accumulation of F-actin mediates tau-induced neuronal degeneration in vivo**. *Nat Cell Biol* 2007, **9**:139-148.

104. Minamide LS, Striegl AM, Boyle JA, Meberg PJ, Bamburg JR: **Neurodegenerative Stimuli Induce Persistent ADF/Cofilin-Actin Rods That Disrupt Distal Neurite Function**. *Nat Cell Biol* 2000, **2**:628-636.

105. Streig AM, Minamide LS, Bamburg JR: **Induction of ADF-actin rods in neurites by mitochondrial inhibition: a common mechanism for neurodegeneration?** *Mol Biol Cell* 1998, **9**:15a.

106. Whiteman IT, Minamide LS, Goh de L, Bamburg JR, Goldsbury C: **Rapid changes in phospho-MAP/tau epitopes during neuronal stress: cofilin-actin rods primarily recruit microtubule binding domain epitopes**. *PLoS ONE* 2011, **6**(6):e20878.

107. Galloway PG, Perry G, Kosik KS, Gambetti P: **Hirano bodies contain tau protein**. *Brain Res* 1987, **403**(2):337-340.

108. Peterson C, Kress Y, Vallee R, Goldman JE: **High molecular weight microtubule-associated proteins bind to actin lattices (Hirano bodies)**. *Acta Neuropathol* 1988, **77**(2):168-174.

109. Munoz DG, Wang D, Greenberg BD: **Hirano bodies accumulate C-terminal sequences of beta-amyloid precursor protein (beta-APP) epitopes**. *J Neuropathol Exp Neurol* 1993, **52**(1):14-21.

110. Hirano A: **Hirano bodies and related neuronal inclusions**. *Neuropathol Appl Neurobiol* 1994, **20**(1):3-11.

111. Spencer PS, Thomas PK: **Ultrastructural studies of the dying-back process. II. The sequestration and removal by Schwann cells and oligodendrocytes of organelles from normal and diseases axons**. *Journal of neurocytology* 1974, **3**(6):763-783.

112. Cavanagh JB, Blakemore WF, Kyu MH: **Fibrillary accumulations in oligodendrogial processes of rats subjected to protocaval anastomosis**. *Journal of the neurological sciences* 1971, **14**(2):143-152.

113. Schmidt ML, Lee VM, Trojanowski JQ: **Analysis of epitopes shared by Hirano bodies and neurofilament proteins in normal and Alzheimer's disease hippocampus**. *Lab Invest* 1989, **60**(4):513-522.

114. Schochet SS, Jr., McCormick WF: **Ultrastructure of Hirano bodies**. *Acta Neuropathol* 1972, **21**(1):50-60.

115. Maloney MT, Bamburg JR: **Cofilin-mediated neurodegeneration in Alzheimer's disease and other amyloidophathies**. *Mol Neurobiol* 2007, **35**:21-44.

116. Maselli AG, Davis R, Furukawa R, Fechheimer M: **Formation of Hirano bodies in Dictyostelium and mammalian cells induced by expression of a modified form of an actin cross-linking protein**. *J Cell Sci* 2002, **115**:1939-1952.

117. Maselli AG, Furukawa R, Thomson SAM, Davis RC, Fechheimer M: **Formation of Hirano bodies induced by expression of an actin cross-linking protein with a gain of function mutation**. *Eucaryot Cell* 2003, **2**:778-787.

118. Lim RWL, Furukawa R, Cartwright RC, Fechheimer M: **Evidence For Three Distinct Actin Binding Sites in the Dictyostelium discoideum 34 kD F-Actin Bundling Protein**. *Mol Biol Cell* 1996, **7**:540a.

119. Lim RWL, Furukawa R, Fechheimer M: **Evidence of intramolecular regulation of the Dictyostelium discoideum 34,000 dalton F-actin bundling protein**. *Biochemistry* 1999b, **38**:16323-16332.

120. Fechheimer M, Taylor DL: **Isolation and characterization of a 30,000-dalton calcium-sensitive actin cross-linking protein from Dictyostelium discoideum**. *J Biol Chem* 1984, **259**:4514-4520.

121. Fechheimer M: **The Dictyostelium discoideum 30,000-dalton protein is an actin filament-bundling protein that is selectively present in filopodia**. *J Cell Biol* 1987, **104**:1539-1551.

122. Fechheimer M, Ingalls HM, Furukawa R, Luna EJ: **Association of the Dictyostelium 30,000 dalton actin bundling protein with contact regions.** *J Cell Sci* 1994, **107**:2393-2401.

123. Furukawa R, Fechheimer M: **Differential localization of alpha-actinin and the 30 kD actin-bundling protein in the cleavage furrow, phagocytic cup, and contractile vacuole of Dictyostelium discoideum**. *Cell Motil Cytoskel* 1994, **29**:46-56.

124. Rivero F, Furukawa R, Noegel AA, Fechheimer M: **Dictyostelium discoideum cells lacking the 34,000 dalton actin binding protein can grow, locomote, and develop, but exhibit defects in regulation of cell structure and movement: a case of partial redundancy.** *J Cell Biol* 1996, **135**:965-980.

125. Lim RWL, Furukawa R, Eagle S, Cartwright RC, Fechheimer M: **Three distinct F-actin binding sites in the Dictyostelium discoideum 34,000 dalton actin bundling protein**. *Biochemistry* 1999a, **38**:800-812.

126. Ha S, Furukawa R, Stramiello M, Wagner JJ, Fechheimer M: **Transgenic mouse model for the formation of Hirano bodies**. *BMC Neurosci* 2011, **12**:97.

127. Ballatore C, Lee VM, Trojanowski JQ: **Tau-mediated neurodegeneration in Alzheimer's disease and related disorders**. *Nat Rev Neurosci* 2007, **8**:663-672.

128. Griffith LM, Pollard TD: **Evidence for actin filament-microtubule interaction mediated by microtubule-associated proteins**. *The Journal of Cell Biology* 1978, **78**(3):958-965.

129. Selden SC, Pollard TD: **Phosphorylation of microtubule-associated proteins regulates their interaction with actin filaments**. *J Biol Chem* 1983, **258**(11):7064-7071.

130. Kotani S, Nishida E, Kumagai H, Sakai H: **Calmodulin inhibits interaction of actin with MAP2 and Tau, two major microtubule-associated proteins**. *The Journal of Biological Chemistry* 1985, **260**(19):10779-10783.

131. Yamauchi PS, Purich DL: **Microtubule-associated protein interactions with actin filaments: evidence for differential behavior of neuronal MAP-2 and tau in the presence of phosphatidyl-inositol**. *Biochemical and Biophysical Research Communications* 1993, **190**(3):710-715.

132. Farias GA, Munoz JP, Garrido J, Maccioni RB: **Tubulin, actin, and tau protein interactions and the study of their macromolecular assemblies**. *Journal of Cellular Biochemistry* 2002, **85**(2):315-324.

133. Correas I, Padilla R, Avila J: **The tubulin-binding sequence of brain microtubule-associated proteins, tau and MAP-2, is also involved in actin binding**. *Biochem J* 1990, **269**(1):61-64.

134. He HJ, Wang XS, Pan R, Wang DL, Liu MN, He RQ: **The proline-rich domain of tau plays a role in interactions with actin**. *BMC Cell Biol* 2009, **10**:81.

135. Yu JZ, Rasenick MM: **Tau associates with actin in differentiating PC12 cells**. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 2006, **20**(9):1452-1461.

136. Maas T, Eidenmuller J, Brandt R: **Interaction of tau with the neural membrane cortex is regulated by phosphorylation at sites that are modified in paired helical filaments**. *The Journal of Biological Chemistry* 2000, **275**(21):15733-15740.

137. Kempf M, Clement A, Faissner A, Lee G, Brandt R: **Tau binds to the distal axon early in development of polarity in a microtubule- and microfilament-dependent manner**. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 1996, **16**(18):5583-5592.

138. Gotz J, Tolnay M, Barmettler R, Chen F, Probst A, Nitsch RM: **Oligodendroglial tau filament formation in transgenic mice expressing G272V tau**. *Eur J Neurosci* 2001, **13**(11):2131-2140.

139. Bunker JM, Kamath K, Wilson L, Jordan MA, Feinstein SC: **FTDP-17 mutations compromise the ability of tau to regulate microtubule dynamics in cells**. *The Journal of Biological Chemistry* 2006, **281**(17):11856-11863.

140. Hong M, Zhukareva V, Vogelsberg-Ragaglia V, Wszolek Z, Reed L, Miller BI, Geschwind DH, Bird TD, McKeel D, Goate A, Morris JC, Wilhelmsen KC, Schellenberg GD, Trojanowski JQ, Lee VM: **Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17**. *Science* 1998, **282**(5395):1914-1917.

141. Lewis J, McGowan E, Rockwood J, Melrose H, Nacharaju P, Van Slegtenhorst M, Gwinn-Hardy K, Paul Murphy M, Baker M, Yu X, Duff K, Hardy J, Corral A, Lin WL, Yen SH, Dickson DW, Davies P, Hutton M: **Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein**. *Nature Genetics* 2000, **25**(4):402-405.

142. Gotz J, Chen F, Barmettler R, Nitsch RM: **Tau filament formation in transgenic mice expressing P301L tau**. *The Journal of Biological Chemistry* 2001, **276**(1):529-534.

143. Tatebayashi Y, Miyasaka T, Chui DH, Akagi T, Mishima K, Iwasaki K, Fujiwara M, Tanemura K, Murayama M, Ishiguro K, Planel E, Sato S, Hashikawa T, Takashima A: **Tau filament formation and associative memory deficit in aged mice expressing mutant (R406W) human tau**. *Proceedings of the National Academy of Sciences of the United States of America* 2002, **99**(21):13896-13901.

144. Gauthier-Kemper A, Weissmann C, Golovyashkina N, Sebo-Lemke Z, Drewes G, Gerke V, Heinisch JJ, Brandt R: **The frontotemporal dementia mutation R406W blocks tau's interaction with the membrane in an annexin A2-dependent manner**. *The Journal of Cell Biology* 2011, **192**(4):647-661.

145. Sydow A, Van der Jeugd A, Zheng F, Ahmed T, Balschun D, Petrova O, Drexler D, Zhou L, Rune G, Mandelkow E, D'Hooge R, Alzheimer C, Mandelkow EM: **Tau-induced defects in synaptic plasticity, learning, and memory are reversible in transgenic mice after switching off the toxic Tau mutant**. *J Neurosci* 2011, **31**(7):2511-2525.

146. Khlistunova I, Biernat J, Wang Y, Pickhardt M, von Bergen M, Gazova Z, Mandelkow E, Mandelkow EM: **Inducible expression of Tau repeat domain in cell models of tauopathy: aggregation is toxic to cells but can be reversed by inhibitor drugs**. *The Journal of Biological Chemistry* 2006, **281**(2):1205-1214.

147. Wang YP, Biernat J, Pickhardt M, Mandelkow E, Mandelkow EM: **Stepwise proteolysis liberates tau fragments that nucleate the Alzheimer-like aggregation of full-length tau in a neuronal cell model**. *Proceedings of the National Academy of Sciences of the United States of America* 2007, **104**(24):10252-10257.

148. Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, Tsuda T, Mar L, Foncin JF, Bruni AC, Montesi MP, Sorbi S, Rainero I, Pinessi L, Nee L, Chumakov I, Pollen D, Brookes A, Sanseau P, Polinsky RJ, Wasco W, Da Silva HA, Haines JL, Perkicak-Vance MA, Tanzi RE, Roses AD, Fraser PE, Rommens JM, St George-Hyslop PH: **Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease**. *Nature* 1995, **375**(6534):754-760.

149. Levy-Lahad E, Wasco W, Poorkaj P, Romano DM, Oshima J, Pettingell WH, Yu CE, Jondro PD, Schmidt SD, Wang K, et al.: **Candidate gene for the**

chromosome 1 familial Alzheimer's disease locus. *Science* 1995, **269**(5226):973-977.

150. Borquez DA, Gonzalez-Billault C: **The amyloid precursor protein intracellular domain-fe65 multiprotein complexes: a challenge to the amyloid hypothesis for Alzheimer's disease?** *Int J Alzheimers Dis* 2012, **2012**:353145.

151. Pardossi-Piquard R, Checler F: **The physiology of the beta-amyloid precursor protein intracellular domain AICD**. *Journal of neurochemistry* 2012, **120 Suppl 1**:109-124.

152. von Rotz RC, Kohli BM, Bosset J, Meier M, Suzuki T, Nitsch RM, Konietzko U: **The APP intracellular domain forms nuclear multiprotein complexes and regulates the transcription of its own precursor**. *J Cell Sci* 2004, **117**:4435-4448.

153. Hutton M, Lendon CL, Rizzu P, Baker M, Froelich S, Houlden H, Pickering-Brown S, Chakraverty S, Isaacs A, Grover A, Hackett J, Adamson J, Lincoln S, Dickson D, Davies P, Petersen RC, Stevens M, de Graaff E, Wauters E, van Baren J, Hillebrand M, Joosse M, Kwon JM, Nowotny P, Che LK, Norton J, Morris JC, Reed LA, Trojanowski J, Basun H, Lannfelt L, Neystat M, Fahn S, Dark F, Tannenberg T, Dodd PR, Hayward N, Kwok JB, Schofield PR, Andreadis A, Snowden J, Craufurd D, Neary D, Owen F, Oostra BA, Hardy J, Goate A, van Swieten J, Mann D, Lynch T, Heutink P: **Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17**. *Nature* 1998, **393**(6686):702-705.

154. Martinez-Saez E, Gelpi E, Rey MJ, Ferrer I, Ribalta T, Botta-Orfila T, Nos C, Yague J, Sanchez-Valle R: **Hirano body-rich subtypes of Creutzfeldt-Jakob disease**. *Neuropathology and applied neurobiology* 2012, **38**(2):153-161.

155. Maciver SK, Harrington CR: **Two actin binding proteins, actin depolymerizing factor and cofilin, are associated with Hirano bodies**. *Neuroreport* 1995, **6**(15):1985-1988.

156. Jordan-Sciutto K, Dragich J, Walcott D, Bowser R: **The presence of FAC1 protein in Hirano bodies**. *Neuropathol Appl Neurobiol* 1998, **24**(5):359-366.

157. Peress NS, Perillo E: **Differential expression of TFG Beta 1, 2, and 3 isotypes in Alzheimer's disease: a comparative immunohistochemical study with cerebral infarction, aged human and mouse control brainsq**. *J Neuropathol Exp Neurol* 1995, **54**:802-811.

158. Li X, Kumar Y, Zempel H, Mandelkow EM, Biernat J, Mandelkow E: **Novel diffusion barrier for axonal retention of Tau in neurons and its failure in neurodegeneration**. *The EMBO Journal* 2011, **30**(23):4825-4837.

159. Higuchi M, Zhang B, Forman MS, Yoshiyama Y, Trojanowski JQ, Lee VM: **Axonal degeneration induced by targeted expression of mutant human tau in oligodendrocytes of transgenic mice that model glial tauopathies**. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2005, **25**(41):9434-9443.

160. Kosik KS, Joachim CL, Selkoe DJ: **Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer**

disease. *Proceedings of the National Academy of Sciences of the United States of America* 1986, **83**(11):4044-4048.

161. Lee VM, Balin BJ, Otvos L, Jr., Trojanowski JQ: **A68: a major subunit of paired helical filaments and derivatized forms of normal Tau**. *Science* 1991, **251**(4994):675-678.

162. Braak E, Braak H, Mandelkow EM: **A sequence of cytoskeleton changes related to the formation of neurofibrillary tangles and neuropil threads**. *Acta neuropathologica* 1994, **87**(6):554-567.

163. Braak H, Braak E: **Neuropathological stageing of Alzheimer-related changes**. *Acta neuropathologica* 1991, **82**(4):239-259.

164. Holzer M, Holzapfel HP, Zedlick D, Bruckner MK, Arendt T: **Abnormally phosphorylated tau protein in Alzheimer's disease: heterogeneity of individual regional distribution and relationship to clinical severity**. *Neuroscience* 1994, **63**(2):499-516.

165. Furgerson M, Fechheimer M, Furukawa R: **Model Hirano Bodies Protect Against Tau-Independent and Tau-Dependent Cell Death Initiated by the Amyloid Precursor Protein Intracellular Domain**. *PLoS ONE, submitted* 2012.

166. Fath T, Eidenmuller J, Brandt R: **Tau-mediated cytotoxicity in a pseudohyperphosphorylation model of Alzheimer's disease**. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2002, **22**(22):9733-9741.

167. Hedgepeth CM, Conrad LJ, Zhang J, Huang HC, Lee VM, Klein PS: **Activation of the Wnt signaling pathway: a molecular mechanism for lithium action**. *Developmental Biology* 1997, **185**(1):82-91.

168. Kinoshita A, Whelan CM, Berezovska O, Hyman BT: **The gamma secretase-generated carboxyl-terminal domain of the amyloid precursor protein induces apoptosis via Tip60 in H4 cells**. *J Biol Chem* 2002, **277**:28530-28536.

169. Summers SA, Kao AW, Kohn AD, Backus GS, Roth RA, Pessin JE, Birnbaum MJ: **The role of glycogen synthase kinase 3beta in insulin-stimulated glucose metabolism**. *The Journal of Biological Chemistry* 1999, **274**(25):17934-17940.

170. Garwood CJ, Pooler AM, Atherton J, Hanger DP, Noble W: **Astrocytes are important mediators of Abeta-induced neurotoxicity and tau phosphorylation in primary culture**. *Cell Death Dis* 2011, **2**:e167.

171. Vogelsberg-Ragaglia V, Bruce J, Richter-Landsberg C, Zhang B, Hong M, Trojanowski JQ, Lee VM: **Distinct FTDP-17 missense mutations in tau produce tau aggregates and other pathological phenotypes in transfected CHO cells**. *Mol Biol Cell* 2000, **11**:4093-4104.

172. Brandt R, Leger J, Lee G: **Interaction of tau with the neural plasma membrane mediated by tau's amino-terminal projection domain**. *The Journal of Cell Biology* 1995, **131**(5):1327-1340.

173. Shahani N, Subramaniam S, Wolf T, Tackenberg C, Brandt R: **Tau aggregation and progressive neuronal degeneration in the absence of changes in spine density and morphology after targeted expression of Alzheimer's disease-relevant tau constructs in organotypic hippocampal slices**. *The Journal of*

neuroscience : the official journal of the Society for Neuroscience 2006, **26**(22):6103-6114.

174. Hundelt M, Fath T, Selle K, Oesterwind K, Jordan J, Schultz C, Gotz J, von Engelhardt J, Monyer H, Lewejohann L, Sachser N, Bakota L, Brandt R: **Altered phosphorylation but no neurodegeneration in a mouse model of tau hyperphosphorylation**. *Neurobiol Aging* 2009.

175. Eidenmüller J, Fath T, Hellwig A, Reed J, Sontag E, Brandt R: **Structural and functional implications of tau hyperphosphorylation: information from phosphorylation-mimicking mutated tau proteins**. *Biochimistry* 2000, **39**:13166-13175.

176. Eidenmuller J, Fath T, Hellwig A, Reed J, Sontag E, Brandt R: **Structural and functional implications of tau hyperphosphorylation: information from phosphorylation-mimicking mutated tau proteins**. *Biochemistry* 2000, **39**(43):13166-13175.

177. Morishima-Kawashima M, Hasegawa M, Takio K, Suzuki M, Yoshida H, Titani K, Ihara Y: **Proline-directed and non-proline-directed phosphorylation of PHF-tau**. *The Journal of Biological Chemistry* 1995, **270**(2):823-829.

178. Lasagna-Reeves CA, Castillo-Carranza DL, Guerrero-Muoz MJ, Jackson GR, Kayed R: **Preparation and characterization of neurotoxic tau oligomers**. *Biochemistry* 2010, **49**(47):10039-10041.

179. de Calignon A, Fox LM, Pitstick R, Carlson GA, Bacskai BJ, Spires-Jones TL, Hyman BT: **Caspase activation precedes and leads to tangles**. *Nature* 2010, **464**(7292):1201-1204.

180. Kremer A, Louis JV, Jaworski T, Van Leuven F: **GSK3 and Alzheimer's Disease: Facts and Fiction**. *Front Mol Neurosci* 2011, **4**:17.

181. Takashima A, Noguchi K, Michel G, Mercken M, Hoshi M, Ishiguro K, Imahori K: **Exposure of rat hippocampal neurons to amyloid beta peptide (25-35) induces the inactivation of phosphatidyl inositol-3 kinase and the activation of tau protein kinase I/glycogen synthase kinase-3 beta**. *Neuroscience letters* 1996, **203**(1):33-36.

182. Roger B, Al-Bassam J, Dehmelt L, Milligan RA, Halpain S: **MAP2c, but not tau, binds and bundles F-actin via its microtubule binding domain**. *Curr Biol* 2004, **14**(5):363-371.

183. Biernat J, Gustke N, Drewes G, Mandelkow EM, Mandelkow E: **Phosphorylation of Ser262 strongly reduces binding of tau to microtubules: distinction between PHF-like immunoreactivity and microtubule binding**. *Neuron* 1993, **11**(1):153-163.

184. Bramblett GT, Goedert M, Jakes R, Merrick SE, Trojanowski JQ, Lee VM: **Abnormal tau phosphorylation at Ser396 in Alzheimer's disease recapitulates development and contributes to reduced microtubule binding**. *Neuron* 1993, **10**(6):1089-1099.

185. Dayanandan R, Van Slegtenhorst M, Mack TG, Ko L, Yen SH, Leroy K, Brion JP, Anderton BH, Hutton M, Lovestone S: **Mutations in tau reduce its microtubule binding properties in intact cells and affect its phosphorylation**. *FEBS Letters* 1999, **446**(2-3):228-232.

186. Connell JW, Gibb GM, Betts JC, Blackstock WP, Gallo J, Lovestone S, Hutton M, Anderton BH: **Effects of FTDP-17 mutations on the in vitro phosphorylation of tau by glycogen synthase kinase 3beta identified by mass spectrometry demonstrate certain mutations exert long-range conformational changes**. *FEBS Letters* 2001, **493**(1):40-44.

187. Jeganathan S, von Bergen M, Brutlach H, Steinhoff HJ, Mandelkow E: **Global hairpin folding of tau in solution**. *Biochemistry* 2006, **45**(7):2283-2293.

188. Harris JA, Devidze N, Halabisky B, Lo I, Thwin MT, Yu GQ, Bredesen DE, Masliah E, Mucke L: **Many neuronal and behavioral impairments in transgenic mouse models of Alzheimer's disease are independent of caspase cleavage of the amyloid precursor protein**. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2010, **30**(1):372-381.

189. Kajiwara Y, Akram A, Katsel P, Haroutunian V, Schmeidler J, Beecham G, Haines JL, Pericak-Vance MA, Buxbaum JD: **FE65 binds Teashirt, inhibiting expression of the primate-specific caspase-4**. *PLoS ONE* 2009, **4**(4):e5071.

190. Noble W, Planel E, Zehr C, Olm V, Meyerson J, Suleman F, Gaynor K, Wang L, LaFrancois J, Feinstein B, Burns M, Krishnamurthy P, Wen Y, Bhat R, Lewis J, Dickson D, Duff K: **Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration in vivo**. *Proceedings of the National Academy of Sciences of the United States of America* 2005, **102**(19):6990-6995.

191. Perez M, Hernandez F, Lim F, Diaz-Nido J, Avila J: **Chronic lithium treatment decreases mutant tau protein aggregation in a transgenic mouse model**. *J Alzheimers Dis* 2003, **5**(4):301-308.

192. Pap M, Cooper GM: **Role of glycogen synthase kinase-3 in the phosphatidylinositol 3-Kinase/Akt cell survival pathway**. *The Journal of Biological Chemistry* 1998, **273**(32):19929-19932.

193. Bijur GN, De Sarno P, Jope RS: **Glycogen synthase kinase-3beta facilitates staurosporine- and heat shock-induced apoptosis. Protection by lithium**. *The Journal of Biological Chemistry* 2000, **275**(11):7583-7590.

194. Zhang F, Phiel CJ, Spece L, Gurvich N, Klein PS: **Inhibitory phosphorylation of glycogen synthase kinase-3 (GSK-3) in response to lithium. Evidence for autoregulation of GSK-3**. *The Journal of Biological Chemistry* 2003, **278**(35):33067-33077.

195. Bardai FH, D'Mello SR: **Selective toxicity by HDAC3 in neurons: regulation by Akt and GSK3beta**. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2011, **31**(5):1746-1751.

196. Vogelsberg-Ragaglia V, Bruce J, Richter-Landsberg C, Zhang B, Hong M, Trojanowski JQ, Lee VM: **Distinct FTDP-17 missense mutations in tau produce tau aggregates and other pathological phenotypes in transfected CHO cells**. *Molecular Biology of the Cell* 2000, **11**(12):4093-4104.

197. Santa-Maria I, Santpere G, MacDonald MJ, de Barreda EG, Hernandez F, Moreno FJ, Ferrer I, Avila J: **Coenzyme Q Induces Tau Aggregation, Tau Filaments, and Hirano Bodies**. *J Neuropathol Exp Neurol* 2008, **67**:428-434.

198. Gomez-Sintes R, Hernandez F, Bortolozzi A, Artigas F, Avila J, Zaratin P, Gotteland JP, Lucas JJ: **Neuronal apoptosis and reversible motor deficit in dominant-negative GSK-3 conditional transgenic mice**. *The EMBO Journal* 2007, **26**(11):2743-2754.

199. Min SW, Cho SH, Zhou Y, Schroeder S, Haroutunian V, Seeley WW, Huang EJ, Shen Y, Masliah E, Mukherjee C, Meyers D, Cole PA, Ott M, Gan L: **Acetylation of tau inhibits its degradation and contributes to tauopathy**. *Neuron* 2010, **67**(6):953-966.

200. Cohen TJ, Guo JL, Hurtado DE, Kwong LK, Mills IP, Trojanowski JQ, Lee VM: **The acetylation of tau inhibits its function and promotes pathological tau aggregation**. *Nat Commun* 2011, **2**:252.

201. Wang Y, Garg S, Mandelkow EM, Mandelkow E: **Proteolytic processing of tau**. *Biochemical Society Transactions* 2010, **38**(4):955-961.

202. Singh TJ, Haque N, Grundke-Iqbal I, Iqbal K: **Rapid Alzheimer-like phosphorylation of tau by the synergistic actions of non-proline-dependent protein kinases and GSK-3**. *FEBS Letters* 1995, **358**(3):267-272.

203. Zempel H, Thies E, Mandelkow E, Mandelkow EM: **Abeta oligomers cause localized Ca(2+) elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines**. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2010, **30**(36):11938-11950.

204. Eidenmüller J, Fath T, Maas T, Pool M, Sontag E, Brandt R: **Phosphorylation-mimicking glutamate clusters in the proline-rich region are sufficient to**

simulate the functional deficiencies of hyperphosphorylated tau protein. *Biochem J* 2001, **357**:759-767.

205. Wilbur JD, Chen CY, Manalo V, Hwang PK, Fletterick RJ, Brodsky FM: **Actin binding by Hip1 (huntingtin-interacting protein 1) and Hip1R (Hip1-related protein) is regulated by clathrin light chain**. *The Journal of Biological Chemistry* 2008, **283**(47):32870-32879.

206. Brett TJ, Legendre-Guillemin V, McPherson PS, Fremont DH: **Structural definition of the F-actin-binding THATCH domain from HIP1R**. *Nat Struct Mol Biol* 2006, **13**(2):121-130.

207. Yoshiyama Y, Higuchi M, Zhang B, Huang SM, Iwata N, Saido TC, Maeda J, Suhara T, Trojanowski JQ, Lee VM: **Synapse loss and microglial activation precede tangles in a P301S tauopathy mouse model**. *Neuron* 2007, **53**(3):337-351.

208. Zhang B, Higuchi M, Yoshiyama Y, Ishihara T, Forman MS, Martinez D, Joyce S, Trojanowski JQ, Lee VM: **Retarded axonal transport of R406W mutant tau in transgenic mice with a neurodegenerative tauopathy**. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 2004, **24**(19):4657-4667.

Table 1: *Relative aggregation properties of tau and cell death*. S = aggregates in solution with recombinant protein, C = aggregates in cell culture, B = aggregates in mouse models of tauopathy. ND = not determined.

Table 1. Relative aggregation properties of tau and cell death						
Tau mutant	***Propensity to aggregate***			***Cell death CT-GFP+tau***	***% protection (of total) CT-GFP+tau+AICD***	***Reference***
	S	***C***	***B***			
352PHP	-	-	-	9%	50%	[147-149]
441 wt	+	-	-	4%	48%	[73, 150, 151]
352 wt	+	-	+	6%	59%	[73, 152]
R5H	+	ND	ND	5%	54%	[82]
R406W	+	-	+	5%	58%	[75, 153]
K18	+	-	-	6%	41%	[75]
G272V	+	ND	+	17%	0%	[75, 137]
P301L	+	+	+	26%	0%	[75, 140, 141, 154]
K18ΔK280	+	+	+	24%	41%	[75, 144-146]

Figure 1: *Immunofluoresence localization of tau and model Hirano bodies*. (A) Point mutations R5H, G272V, P301L, and R406W were created in the 2N4R (441) tau isoform. Arrows represent boundaries of K18 (a.a. 244-372)[146]. N1 and N2 represent N-terminal repeats 1 and 2. R1, R2, R3, and R4 represent microtubule binding domain repeats 1-4. Yellow circles designate 10 serine/threonine to glutamic acid mutations that occur in 352PHP tau [172]. This mutant was created in the 352 tau isoform lacking N1, N2, and R2. (B) H4 cells were transiently transfected with FLAG-tagged tau constructs and CT-GFP to induce model Hirano bodies (green). 24 h post-transfection, cells were fixed and stained for good preservation of F-actin. Tau was stained with anti-FLAG primary antibody and Alexa Fluor350 secondary antibody (blue). F-actin was stained using TRITC-phalloidin (red). All tau isoforms colocalize with model Hirano bodies except 352PHP tau, which is localized diffusely throughout the cell. Scale bar = 20 μm.

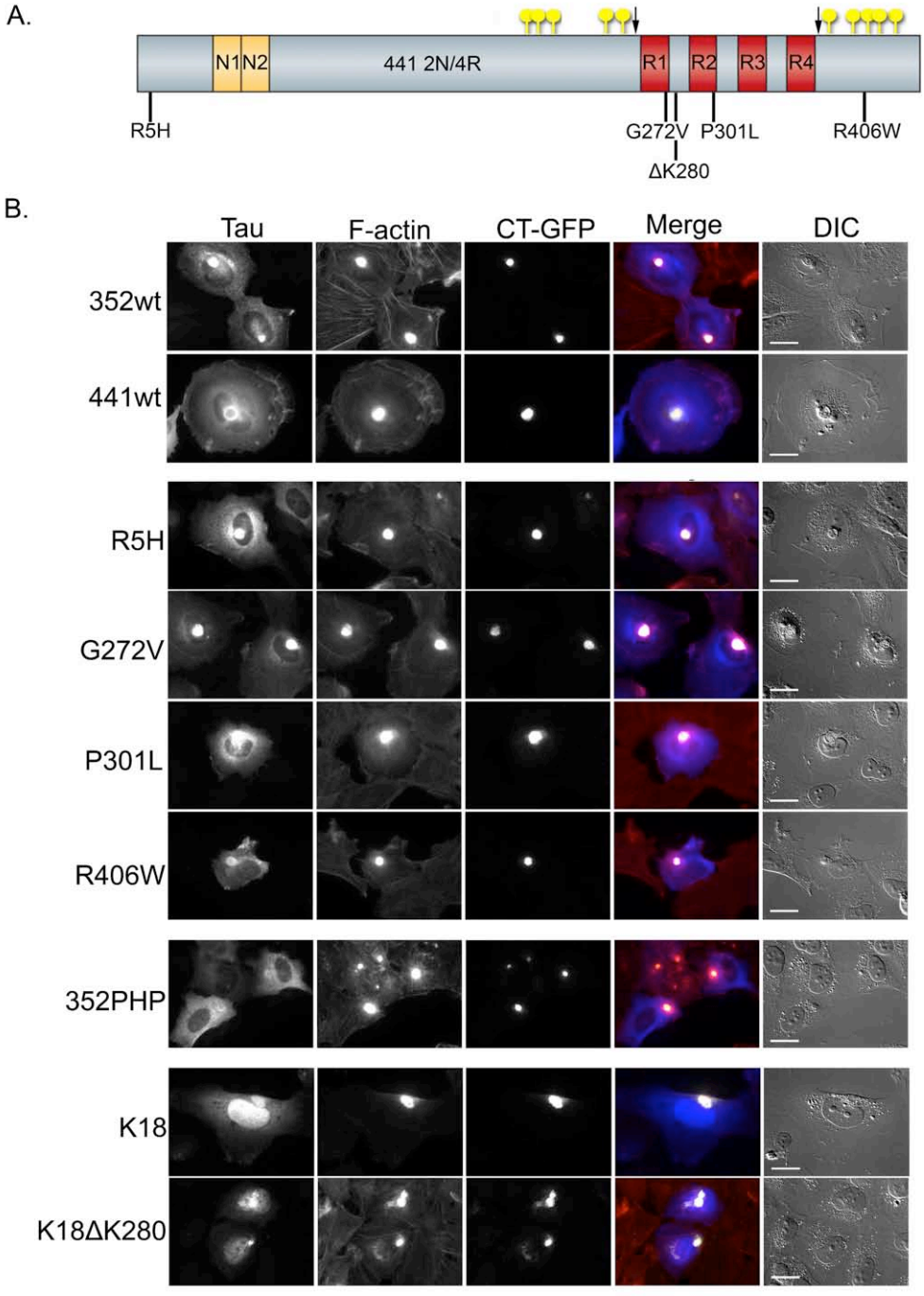

A.
N1
N2
441 2N/4R
R1
R2
R3
R4
R5H
G272V
P301L
R406W
ΔK280
B.
Tau
F-actin
CT-GFP
Merge
DIC
352wt
441wt
R5H
G272V
P301L
R406W
352PHP
K18
K18ΔK280

Figure 2: *FTDP-17 tau and model Hirano bodies differentially modulate cell death.* H4 cells were transiently transfected with equal amounts of plasmid DNA encoding AICD and/or tau in the presence (check, stripe bars) or absence (gray, black bars) of model Hirano bodies (CT-GFP). Model Hirano bodies protect from cell death induced by AICD and/or tau, except in tau mutants G272V, P301L, and K18ΔK280. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Error bars represent the standard deviation.

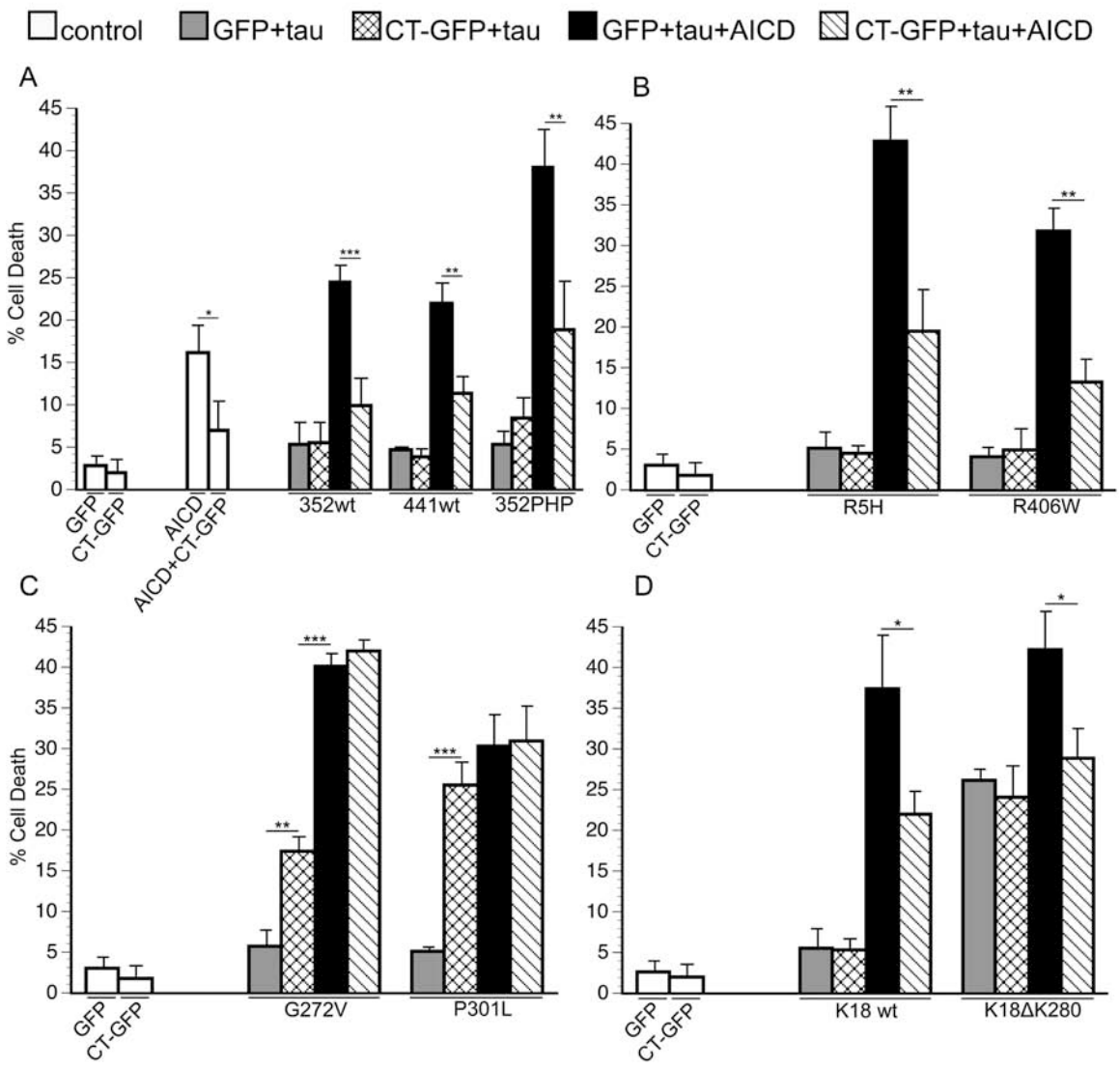
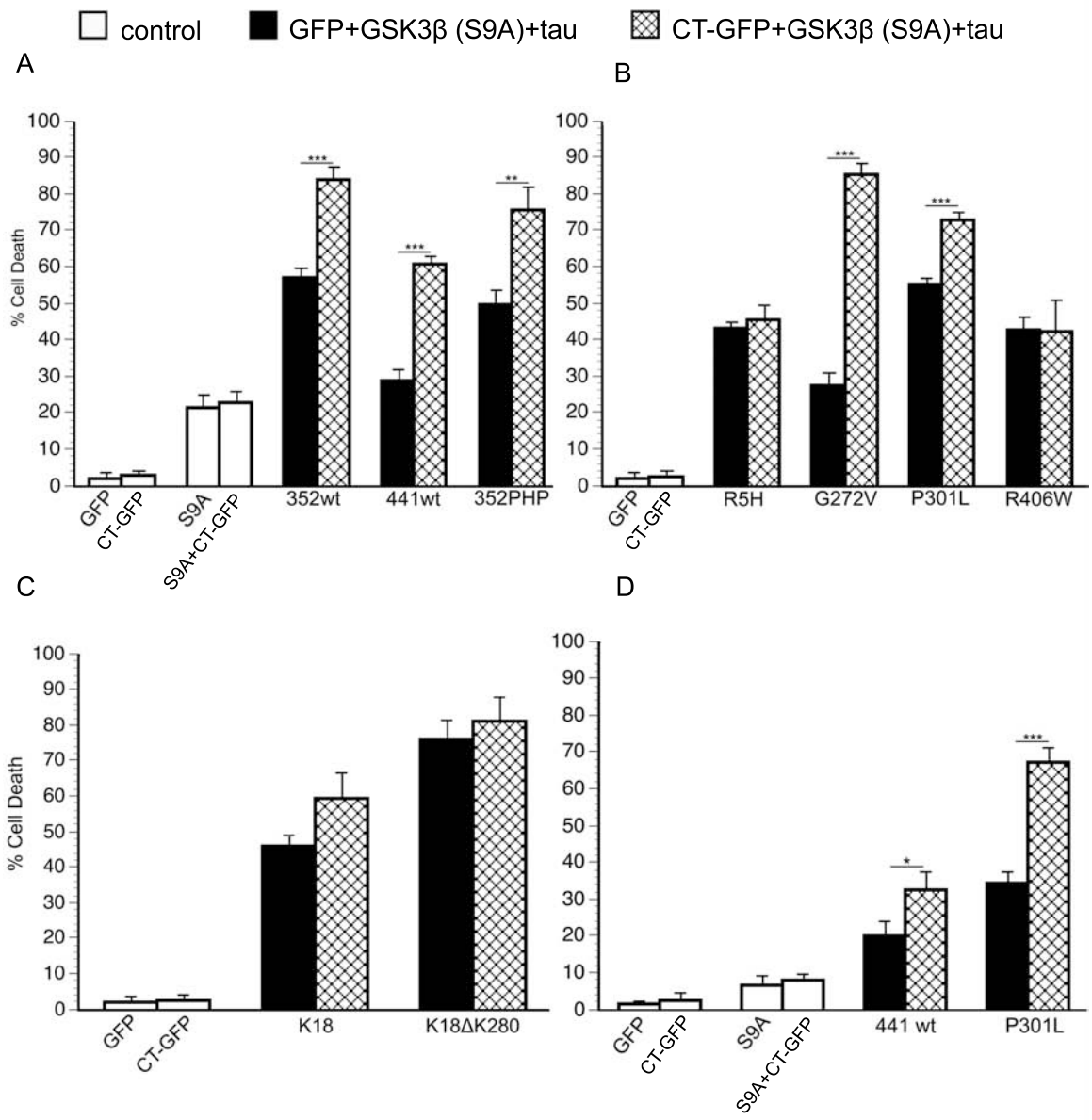

control
GFP+tau
CT-GFP+tau
GFP+tau+AICD
CT-GFP+tau+AICD
A
B
C
D
% Cell Death
GFP
CT-GFP
AICD
AICD+CT-GFP
352wt
441wt
352PHP
R5H
R406W
G272V
P301L
K18 wt
K18ΔK280

Figure 3: *Model Hirano bodies differentially influence cell death in the presence of GSK3β and tau.* H4 cells were transiently transfected with equal amounts of plasmid DNA encoding constitutively active GSK3β (S9A) and tau (black bars) or GSK3β (S9A), tau, and model Hirano bodies (check bars). Model Hirano bodies significantly promote cell death induced by GSK3β (S9A) and tau except in tau mutants R5H, R406W, K18, and K18ΔK280, where they have no effect. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Error bars represent the standard deviation.


control
GFP+GSK3β (S9A)+tau
CT-GFP+GSK3β (S9A)+tau
A
B
C
D
% Cell Death
GFP
CT-GFP
S9A
S9A+CT-GFP
352wt
441wt
352PHP
R5H
G272V
P301L
R406W
K18
K18ΔK280
441 wt