An Investigative Analysis of Underlying Alzheimer's Disease Pathology

Understanding the Pathology of Neurofibril Tangles

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Abstract

Researchers familiar with Alzheimer's Disease (AD) refer to two common pathological hallmarks: β-amyloid plaques and Neurofibril Tangles (NFTs). The development of these pathologies are the leading theory to explain the cause of cognitive decline and dementia-like symptoms of individuals with AD. The literature supports the theory that NFTs are caused by the hyperphosphorylation of the precursor tau protein. However, the mechanism that causes this hyperphosphorylation is still unknown. This review seeks to evaluate the normal function of tau then analyze and expound upon the current research concerning the underlying mechanisms for the hyperphosphorylation of tau observed in Neurofibril Tangles pathologies. This will provide an additional resource for researchers looking to better understand the causation of NFTs, which can aid in the development of therapeutic agents for individuals with AD.

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Introduction

Background: Neurofibril Tangles and Alzheimer's Disease

Alzheimer's Disease (AD) is a common neurodegenerative disease that is prevalent among older populations across all cultures. AD is the sixth leading cause of death in the United States and fifth leading cause of death for individuals over the age of 65. Official death records state that 122,019 individuals died from AD in 2018. AD accounts for 60-70% of recorded dementia, language problems, inability to learn, etc. (Journal of the Alzheimer's Association, 2020). With this prevalence, it is pivotal to aid in the research for Alzheimer's Disease cures and symptom alleviations. But to do this, it is crucial to understand the underlying mechanisms that cause Alzheimer's Disease.

Two primary cellular abnormalities are found in individuals with Alzheimer's Disease. The first feature is the buildup of β-amyloid plaques. Studies have shown that when these plaques are injected into organisms, such as rats or primates, similar pathological signs such as memory loss and cognitive decline can be found (Forny-Germano et al., 2014). The second and equally prominent feature of AD is the buildup of Neurofibril Tangles (NFTs). The buildup of NFTs has also been directly linked to AD symptomatology in similar experimentation (Peter T. Nelson & Irina Alafuzoff, 2012). Furthermore, the research suggests that the buildup of NFTs can have cytotoxic effects on cellular mechanisms, which can impair cell function; this results in cell death and brain atrophy seen in patients with AD.

Given the copious evidence supporting the link between the buildup of Beta Amyloid (Aβ) Plaques and NFT's in AD symptomatology, understanding the mechanisms that induce this

buildup is crucial for identifying treatments and possible cures for AD. In this investigation, the underlying mechanism of Neurofibril Tangle build up will be evaluated and compared to other pathological mechanisms, like Aβ Plaques. This will aid researchers in the development of possible treatments plans and preventative measures for individuals with AD.

Overview of Tau

MAPs (Microtubule associated proteins) were initially discovered as intracellular proteins that stabilize microtubules; however, the increasing number of discovered MAPs have led researchers to believe that they hold a much wider array of functions. Since the 1960s, scientists have now discovered over 20 different MAPs in the mammalian species with a wide array of functions. MAPs are now categorized into five groups based on their function: motor proteins that generate force, enzymes which depolarize microtubules, microtubule nucleators, end binding proteins, and structural proteins (Satish Bodakuntla, A.S Jijumon, Cristopher Villablanca, Christian Gonzalez Billault, & Carsten Janke, 2019). For the purposes of this review, we will study the function of tau, a structural MAP, that has been proven to an important role in the progression of many neurodegenerative diseases.

Tau is a MAP that plays a particularly important role in motor protein regulation, axonal stabilization, and many other pathways during neuronal development. It was initially discovered in 1975 by Dr. Weingarten and was found to be required for the tubulin dimers to assemble into microtubules *in vitro* (M D Weingarten, A H Lockwood, S Y Hwo, & M W Kirschner, 1075). Tau is encoded by a single gene, *mapt*, located in humans on chromosome 17. *Mapt* is transcribed into six different isoforms with three or four microtubule binding domains depending on if exon 10 is present after the post transcriptional modifications, this is also known as alternative splicing reactions. The longest tau isoform, N24R Tau, which contains exon 1, 2, 3, 4,

5, 7, 9, 10, 11, 12, and 13, is typically chosen as the best mammalian model of tau; it has 441 amino acid residues making it the easiest isoform to work with given its size. The primary structure is arranged with an N terminal region made up of 2 N terminal repeats, a proline rich region, 4 microtubule binding domains, and a C terminal domain (Yuxing Xia, Stefan Prokop, & Benoit I. Giasson, 2021). Of these 441 amino acids found in the primary structure of tau, 81 are serine or threonine residues (see figure 1). This high number of serine and threonine residues provide abundant phosphorylation sites on the protein's primary structure, which can alter its function (see figure 1) (Biomolecular Structure, Dynamics, and Interactions Lab, 2015). Furthermore, despite tau being previously considered an intrinsically disordered protein, it has been found to gain secondary structure after post translational modifications like phosphorylation, acetylation, and methylation (Jesus Avila et al., 2016) which suggests the structural requirement of these modifications are required for proper tau function. Tau's unique structure allows it to play an important regulatory role on various cellular functions.

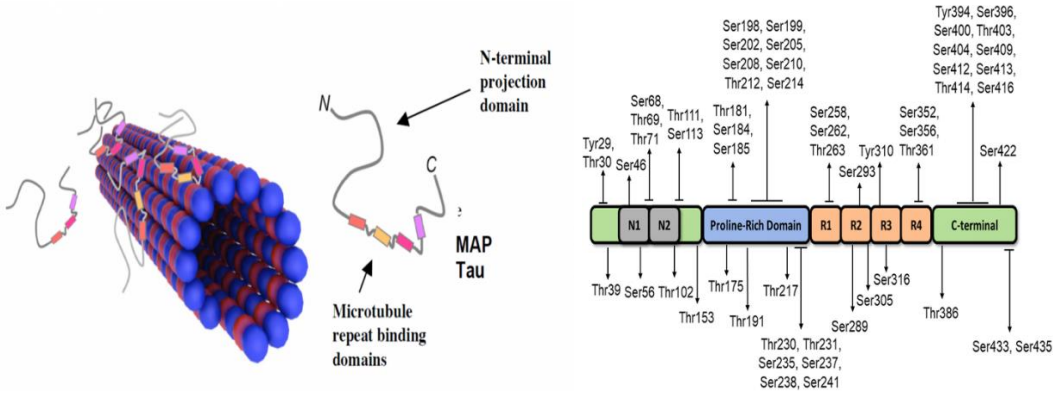

Figure 1. *Tau Phosphorylation Sites. (left) Normal phosphorylated tau binding to microtubule through microtubule repeat binding domains, which stabilizes structure. (right) The phosphorylation sites consisting of copious serine and threonine residues, allowing for proper phosphorylation of tau to enable microtubule stabilization.*

Normal Physiology of Tau in Cellular Transport

Cell transport is a fundamental biological concept that is necessary for cell growth and development. The basic composition of this framework consists of a cell scaffolding, known as microtubules, and various motor proteins for transport. This provides a mechanism to transport proteins and other organelles throughout the cell and to aid in various cellular responses to maintain homeostasis. Understanding the makeup of these cellular structures and how they relate to the normal function of tau will be essential in exploring the pathophysiology associated with tau and AD.

Normal Motor Protein Regulation Through Tau

The two primary motor proteins that transport these vesicles throughout the cell are Kinesin and Dynein. Both motor proteins utilize a tethering mechanism that actively transports cargo through ATP hydrolysis. Kinesin is primarily found to be involved with anterograde transport or transporting cargo to the cell periphery. This is done through a unidirectional stepping mechanism allowing for transport only to the positive end of the microtubule. Dynein motor proteins are more commonly found to be involved in retrograde transport or transport to the center of the cell (see figure 2). Moreover, Dynein has the capacity of bidirectional stepping, which can provide additional transport functions dependent on ATP availability in the cell (Arne Gennerich, Andrew P Carter, Samara L Reck-Peterson, & Ronald D Vale, 2007).

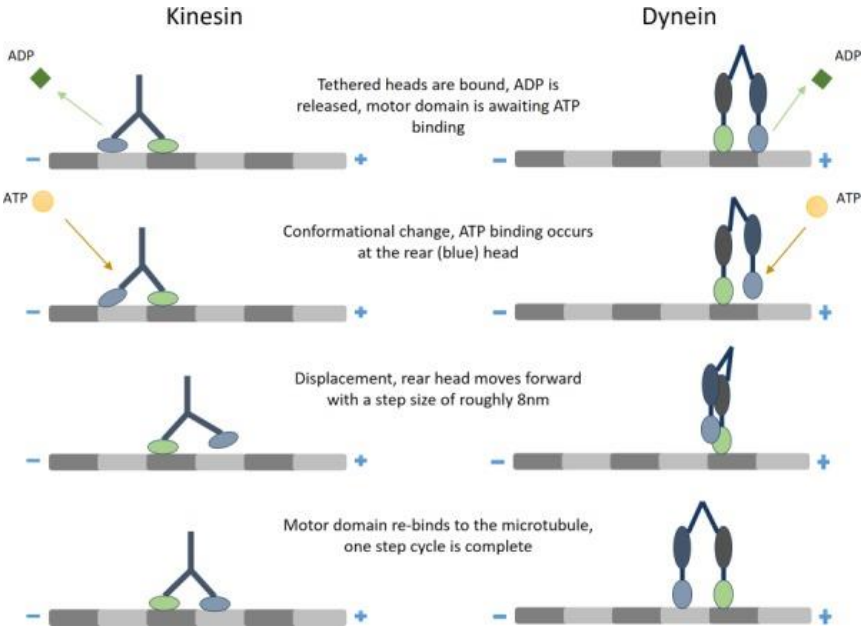

Figure 2. *Intracellular transport along a microtubule. (left) Unidirectional kinesin motor protein undergoing anterograde transport through ATP-dependent tethering mechanism. (right) Bidirectional dynein motor protein undergoing retrograde transport through ATP-dependent tethering mechanism* (Zachary Abraham, Emma Hawley, Daniel Hayosh, Victoria A. Webster-Wood, & Ozan Akkus, 2018).

Tau has been identified to normally regulate various aspects of both Kinesin and Dynein transport along microtubules. A 2018 study found that tau has the capacity to spatially modulate specific motors on Kinesin and Dynein. This can provide a regulatory mechanism in which tau can control the intracellular transport of organelles throughout the cell by altering motor proteins affinity to microtubules. The study also found that tau preferentially upregulates bidirectional transport toward the minus end of the microtubule, which presents another important mechanism from which tau can be utilizes to change intracellular transport patterns. Furthermore, the study noted that as the levels of tau fluctuate, it can begin to act as a direct obstacle by blocking the

motor proteins from traversing the microtubules (Chaudhary, Berger, Berger, & Hendricks, 2018). Other studies have shown that tau can also detach organelles that are being transported by Kinesin (A. Ebneth, R. Godemann, K. Stamer et al., 1998). These mechanisms provide other normal regulatory roles that tau plays in the intracellular transport of materials and organelles via the motor proteins Kinesin and Dynein. However, further research must be done to explore how the cell utilizes these functions of tau to regulate intracellular transport.

Microtubule Stabilization Through Tau

The cell scaffolding is made up of microtubules that radiate throughout the cell providing the pathways for vesicular transport. These microtubules are made up of a tubulin dimer consisting of α tubulin and β tubulin subunits. These tubulin dimers assemble into 13 protofilaments in a head-to-tail manner centered around a hollow core forming a microtubule. Microtubules have the capacity to extend throughout the cytosol through a process known as microtubule polymerization. Furthermore, microtubules are polar in nature which allows them to have both a positively charged, growing end and a negatively charged, dissociating end. Tubulin dimers associated with GTP have the capacity to bind to the positively charged end of the microtubule which allows it to extend forward. As the microtubule grows, the tubulin dimers closer to the negative end of the microtubules begin to dissociate as the GTP is hydrolyzed to GDP. This process is known as treadmilling and creates a dynamic instability that allows microtubules to extend through the cell. If the rate of GTP-bound tubulin dimers binding to the positive end of the microtubule is greater than the rate of GDP tubulin dimer dissociation at the negative end, then the microtubule will grow. However, if the rate of dissociation is greater than the rate of addition, the microtubule will shrink (see figure 3).

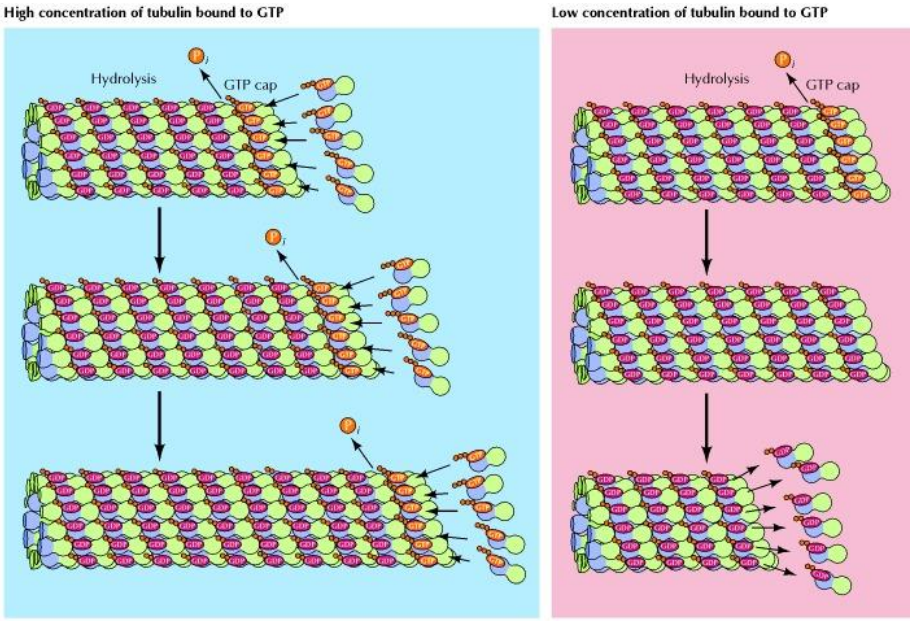

Figure 3. *Microtubule Polymerization. (left) The microtubule has a higher rate of tubulin addition compared to GTP hydrolysis, so it will increase in length. (right) The rate of GTP hydrolysis is greater than the rate of tubulin addition, so it will decrease in length.*

Given this dynamic nature, microtubule polymerization is tightly regulated by microtubule associated proteins (MAPs) including MAP1, MAP2, and the primary protein of this study, tau. Understanding the normal tau-mediated mechanisms of microtubules polymerization will be essential in understanding the normal physiology of tau. Researchers have found that phosphorylation of tau, to a certain degree, is essential for it to carry out its typical cellular function. Phosphorylation at certain serine and threonine residues allows tau to associate with binding domains of microtubules; this stabilizes both the growing and dissociating ends of the polymerization process (Trinczek, Biernat, Baumann, Mandelkow, & Mandelkow, 1995). Thus, the microtubules will be longer and more prone to the transport of intracellular materials. Interestingly, tau has been proven to not only stabilize microtubules during polymerization, but also induce the formation of microtubules. A 2016 study found that when tau was introduced *in*

vivo and *in vitro* with proper intracellular components, it was able to induce the formation of tubulin rings (Kutter, Eichner, Deaconescu, & Kern, 2016). Therefore, tau also plays important regulator roles in the stabilization of microtubules through phosphorylation and the promotion of microtubule formation in cells.

Exploring the Dysregulation of Tau

Hyperphosphorylation of tau has been proven to be a primary causative factor for the NFTs found in individuals with Alzheimer's Disease. Researchers have identified over 20 phosphorylation sites on tau which have a significant correlation to the onset of AD (Bennecib, Gong, Grundke-Iqbal, & Iqbal, 2000). However, researchers have struggled to quantify how much phosphorylation is considered hyperphosphorylation. In a normal human brain, there are typically 2-3 moles of phosphates per mole of tau; researchers have found that the phosphorylation levels in tau are up to 3-4 times greater in individuals with AD (Gong, Liu, Grundke-Iqbal, & Iqbal, 2005). This finding has led many researchers to evaluate the post translational modifications of tau regarding phosphorylation levels.

Hyperphosphorylation of Tau is primarily attributed to two primary causes. The first cause is through mechanisms involving the upregulation of tau kinases. This will increase phosphorylation of the serine and threonine residues in tau which causes tau proteins to clump together in the cell. There have been multiple previously proven kinases which upregulate tau phosphorylation leading to the pathological signs we see in the brain of AD patients. The kinases that will be examined in the review include glycogen synthase kinase 3(GSK-3), cyclin dependent kinase-5(CDK-5), and AMP activated protein kinase (AMPK). This review will evaluate the normal function and the pathophysiology of these kinases as it relates to the hyperphosphorylation of tau.

Positive Regulators of Tau Activity

GSK-3

Glycogen Synthase Kinase (GSK-3) is a common kinase throughout the cell that is responsible for various functions. It was originally found to be an inhibitor of glycogen synthase via phosphorylation and has since been found to be a key regulator of tau phosphorylation (Medina & Wandosell, 2011). It is comprised of two primary isoforms: GSK-3 α and GSK3 β, composed of 483aa and 433aa, respectively. These proteins are known as proline dependent kinases (PDK), which requires a proline residue to initiate phosphorylation of an amino acid. This will play an important role in tau phosphorylation as nearly half of all serine and threonine residues on tau require phosphorylation via a PDK (Bennecib et al., 2000).

The regulation of GSK-3 has been studied extensively since its link to the hyperphosphorylation of tau seen in NFTs. It has been proven to be regulated by post translational modifications, protein complexes, substrate priming, and cellular trafficking. Recent studies have found that phosphorylation of the serine residue at position 21 and position 9 in GSK-3α and GSK-3β respectively, leads to their deactivation (Xianjun Fang et al., 2000). Moreover, a 2011 study suggested as phosphorylation of other residues of GSK-3β increase, the likelihood of phosphorylation of the Serine 9 residue also increases (Medina & Wandosell, 2011). This suggests a cooperative nature of GSK-3, which could have a profound impact on its activity. If there is a slight downregulation of the phosphorylation of GSK-3, this could inhibit other serine and threonine residues from being phosphorylated, which could cause a chain reaction, resulting in the upregulation of GSK-3. Therefore, understanding different mechanisms that can induce phosphorylation of GSK-3α and GSK-3β at Serine 21 and Serine 9 respectively,

can aid in researcher's understanding of how GSK-3α and GSK-3β are upregulated in individuals with AD.

One study has suggested a possible synergistic mechanism describing the phosphorylation of the previously mentioned serine residues. The study initially established that protein kinase A (PKA) has the capacity to inhibit GSK-3 both *in vitro* and *in vivo* through the direct phosphorylation of the previously mentioned inhibitory serine residues. The study also noted that IGF-1 has the capacity to inhibit a PI3K/Akt dependent pathway, which also has the capability of phosphorylating GSK-3 at the previously mentioned Serine residues in response to changes in the energy requirements of the environment (see figure 5) (Marc Delcommenne et al., 1998). The researchers concluded that although these two independent pathways may stimulate the phosphorylation and subsequent inhibition of GSK3, they seem to synergistically coregulate the activity of GSK3, dependent on the environmental energy context. This suggests that a possible defect causing downregulation of the PKA or the Akt dependent pathways could result in increased GSK-3 activation, which could increase the likelihood of tau hyperphosphorylation.

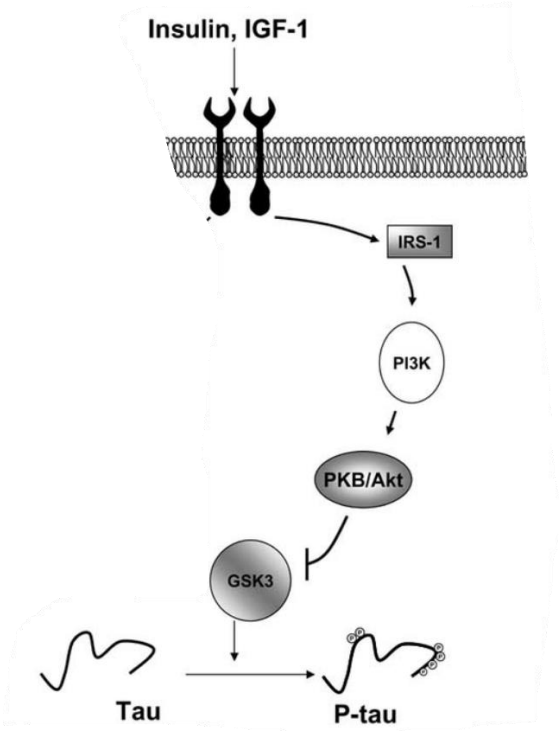

Figure 4. *AKT-dependent signaling pathway. In the presence of IGF-1, a signaling cascade begins with the activation of IRS-1. This activates PI3K which phosphorylates and deactivates Akt, also known as PKB. With Akt inhibited, GSK-3 will be hyperactivated resulting in the up-regulation of the phosphorylation of tau* (Alberto Gomez-Ramos et al., 2006).

Furthermore, understanding possible mechanisms to increase the activation of Akt could provide a method to increase phosphorylation on GSK-3, inhibiting it. Given that Akt signaling pathways have been proven to decrease the activity of GSK-3β, researchers have cross referenced other AD pathologies to find connections to other upstream factors contributing to downregulation of the Akt dependent pathway. Interestingly, Aβ, another precursor protein to AD, has been found to inhibit the previously mentioned Akt pathway in rat hippocampus cultures (Takashima, 2006). This suggests an interconnected relationship between Aβ and tau through Akt activation pathways. However, further research must still be done to better understand the mechanisms which link these two AD pathologies.

Cdk-5

Cyclin Dependent Kinases (CDKs) are a group of kinases that have the ability to phosphorylate Serine and Threonine through interactions with cyclins. CDK-5 is a known kinase that is essential during neuronal development, synaptic plasticity, neurotransmitter release, neuron migration, and development of the CNS (Chackalamannil, Rotella, & Ward, 2017). *In vitro*, CDK-5 has also been shown to protect neurons from cell death by activating the anti-apoptotic protein Bcl-2 and inhibiting ERK1/2 activation in PC12 cells (Kanungo, Zheng, Amin, & Pant, 2009). It has been proven to require both a p35 and p39 substrate to function normally. Furthermore, the CDK-5/p35 complex has also been shown to associate closely with microtubules more than other CDKs, making it a prime candidate for tau pathology research.

CDK5 dysregulation has been associated with various neurodegenerative disease. The primary causative factor found to enhance this dysregulation is the conversion of the p35 substrate to the p25 substrate. This conversion can cause a buildup of p25 in brain tissues which correlates with the pathology seen in individuals with AD. Furthermore, p35 has been shown to increase CDK5 activity without any notable increase in tau phosphorylation; however, the p25/CDK5 complex was shown to increase tau phosphorylation in 3xTG-AD mice models (Piedrahita et al., 2010). Understanding possible mechanisms that increase the conversion of p35 to p25 could be helpful in identifying how to fix the hyperphosphorylation of tau through p25/CDK-5 complex mechanisms.

Interestingly, a group of researchers was testing to see if other AD pathologies could play an effect on the p35 conversion to p25. They found that having increased levels of ischemia and excitotoxicity increase the rate of conversion of p35 cleave to p25 through a calpain regulated mechanism (Kwon et al., 2000). Furthermore, there was an increase in the amyloid β-peptide,

Aβ1–42, as levels of p25/CDK5 complexes increased (see figure 4). This provides another mechanism from which Aβ Plaques and NFTs may be related: the regulation of the p35 substrate in CDK-5 through calpain. However, further research must be done to evaluate how higher rate of cleavage of p35 to p25 via calpain increased the concentration of Aβ1–42 in primary cortical neurons.

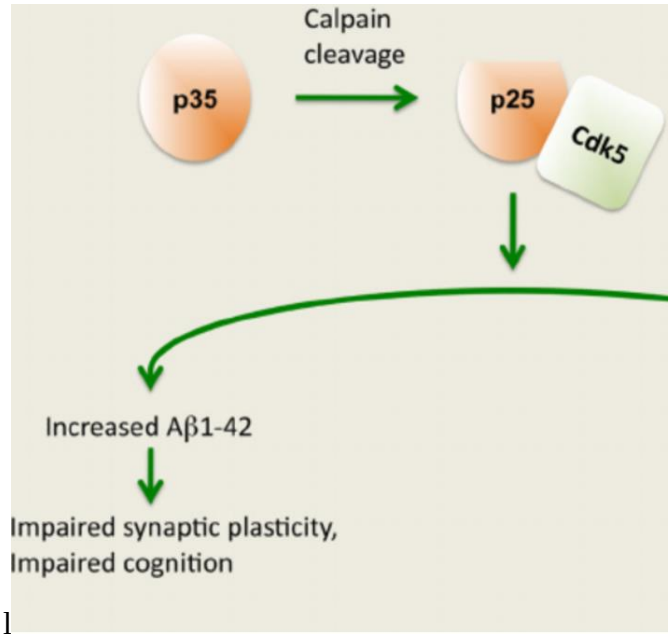

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Figure 4. *Calpain Cleavage of CDK5/p25 Complex. Calpain induces cleavage of p35 to p25, which causes CDK5/p25 to form at higher rates resulting in increased Aβ1–42 levels in cortical brain neurons. This mechanism results in many well-known AD symptoms like limited synaptic plasticity and impaired cognitive function* (Pimplikar, Nixon, Robakis, Shen, & Tsai, 2010).

AMPK

AMPK is kinase generally known to regulate cellular energy levels and consists of two catalytic α subunits and two regulatory β and γ subunits. In this context, it phosphorylates key enzymes in metabolic pathways and regulates transcription by phosphorylating transcription

factors and cofactors. When cellular energy levels are low, AMPK activates glucose and fatty acid uptake and oxidation to increase energy levels (Kim, Yang, Kim, Kim, & Ha, 2016). More recent studies have found that AMPK may play a regulatory effect on microtubules by phosphorylating various serine and threonine residues of tau.

AMPK has previously been found to phosphorylate tau in vitro, causing abnormal accumulation of pre-tangle bearing neurons in all major taupathologies (Vingtdeux, Davies, Dickson, & Marambaud, 2011). This has led researchers to investigate the possible mechanisms of AMPK phosphorylation found in tau pathologies. A more recent study has found that AMPK was able to regulate endogenous tau phosphorylation in vivo in mouse brains (Domise et al., 2016). Therefore, there must be a link between energy metabolism and regulation of tau that must be further evaluated.

One theory suggests that AMPK may also play a detrimental effect in the maintenance of synaptic integrity during times of energetic distress. During energetic stressors, researchers have observed hyperactivation of AMPK, which caused the elimination of post synaptic proteins; this results in induced synaptic loss and neuronal network dysfunction (Domise et al., 2019). Interestingly, a well-known energetic stressor of AMPK is that of excess insulin secretion, and a study from 2005 found that insulin resistance syndrome has been correlated with increased levels of Aβ and other inflammatory agents (Craft, 2005). This suggests yet another link between the two main pathologies of AD. However, further research must still be done to understand how increasing insulin resistance leads to higher levels of Aβ in the cytoplasm of neurons.

Negative Regulators of Tau Activity

The second, and equally likely factor, affecting the hyperphosphorylation of tau is mechanisms involving the downregulation of tau phosphatases, which aid in the

dephosphorylation of tau. When these proteins are downregulated, there will be an increased level of phosphorylated sites on tau because the phosphatases are not removing the phosphate groups at as high of a rate. This would also cause the clumping of tau seen in AD and other tau pathologies. Some previously proven tau phosphatases include Protein Phosphatase 2A (PP2A), Protein Phosphatase 2B(PP2B), and Protein Phosphatase 1(PP1). This review will also evaluate the normal and pathophysiology of these phosphatases as it relates to tau hyperphosphorylation in AD pathology.

PP2A

PP2A is a well-known serine/threonine protein phosphatase in eukaryotic cells. It has many subunits and isozymes that are encoded by various genes to form copious holoenzymes. PP2A serves many cellular functions including cell cycle regulation, cell proliferation, development, and regulation of many multi-signal transduction pathways (Xu, Chen, Zhang, Jeffrey, & Shi, 2008). PP2A was found to represent approximately 71% of phosphatase activity in the human brain, which further emphasizes the importance of understanding its normal physiology and its pathophysiology.

More importantly for this review, PP2A is known to be the most abundant tau phosphatase in the human brain. One study which utilized metabolically active forebrain slices of mice found that after applying okadaic acid, a PP2A inhibitor, to the mice forebrain, there were higher levels of tau phosphorylation at Ser 198, Ser 199, Ser 202, Ser396, and Ser 422; these sites have been linked to higher levels of NFTs after phosphorylation (Bennecib et al., 2000). They reviewed PP2A levels in AD brains against age matched controls and found a 20% decrease in PP2A in the AD brains. Furthermore, this study also found that treatment with PP2A has also decreased the abnormal hyperphosphorylation of tau in NFTs. However, researchers are

still unsure if this decrease was due to direct dephosphorylation of tau, or dephosphorylation that inhibited a kinase that phosphorylates tau. Regardless, PP2A may prove to be a beneficial therapeutic agent when treating AD and other tau pathologies.

Interestingly, a similar study from 2010 found that when PP2A levels in metabolically active brain tissue are lower, the levels of GSK-3β also decrease. PP2A was found to regulate the dephosphorylation of the Serine 9 residue of GSK-3β, which normally inhibits its function. Therefore, the decreased activity of PP2A may have conflicting effects on the phosphorylation of tau. Lower levels of PP2A decrease the rate of dephosphorylation of tau directly, but it also decreases the rate of dephosphorylation of a potential phosphorylator of tau, GSK-3β. In doing this, it effectively amplifies and inhibits the rate of hyperphosphorylation of tau. A 2015 study conveys similar results in that PP2A activation dephosphorylates AMPK, which increases its activation similarly to GSK-3β (Zaha & Young, 2012). Therefore, the holistic effect PP2A has on tau hyperphosphorylation requires further research to differentiates the individual effects of these pathways.

Scientists have struggled to identify the cause for lower levels of PP2A in the brain of AD patients. However, A group of researchers found a possible answer for this. The group conducted a study to analyze the effect that the ApoE 4 allele had on the transcription of the PP2A. The study found that having the ApoE 4 allele negatively impacts the production of PP2A through two mechanisms. ApoE transcriptionally represses the PP2A regulatory subunit and triggers demethylation of the PP2A catalytic subunit which disrupts the catalytic and regulatory subunit complexes. This disruption results in a decreased availability of functional PP2A in the brain of individuals with the ApoE 4 allele (Theendakara, Bredesen, & Rao, 2017). This gene

could present to be a possible target for gene therapy; however, further research must be done to further evaluate the ApoE 4 alleles effect on tau phosphorylation

PP2B

PP2B, also called calcineurin, is another Serine/Threonine phosphatase that consists of 2 subunits: a 60 kDa alpha subunit and a 20 KDa beta subunit. Like PP2A, PP2B plays an important role in various cellular processes including, regulation of the cell cycle, meiosis, mitosis, development and differentiation, metabolism, and apoptosis (Fraga et al., 2010). Furthermore, a previous study demonstrated that PP2B has the capacity to dephosphorylate tau *in vitro* but was unable to determine the effects in vivo (Gong et al., 2005).

PP2B has also been found to carry out import cellular functions involving the assembly of microtubules for intracellular transport. A later study utilizing human biopsied AD brain tissue found that when PP2B was introduced into freshly biopsied tissue, it increased the levels of dephosphorylation of tau found in the samples, which increased the competency of microtubule assembly and stabilization as a whole. (Garver, Lehman, & Billingsley, 1996). The study also postulated that the level of phosphorylation was more dependent on relative equilibrium concentrations between PP2B and other phosphatases to kinases, and this relative concentration differential could affect the assembly of microtubules as a whole (Garver et al., 1996).

Interestingly, another study from USC analyzed the effect of *DSCRI*, a gene localized in chromosome 21 of the human genome, on the regulation of PP2B. The study noted that increased levels of *DSCRI* activation diminishes PP2B activity (Ermak, Morgan, & Davies, 2001). Furthermore, the study reported that with the decrease in PP2B activity, there was an increase in tau hyperphosphorylation. This suggests that overexpression of the gene coding for the PP2B inhibitor, *DSCRI,* may play an important role in the development and onset of NFTs in

Alzheimer's Pathology. However, further research must still be done to evaluate the possible causes of increased *DSCRI* activation.

PP1

PP1 is another serine/threonine phosphatase that plays many important roles in the normal physiology of the cell. PP1 regulates glycogen metabolism, cell cycle progression, and muscle relaxation (Kerff, Langsetmo, Tao, Dominguez, & Terrak, 2004). There are many variations of the PP1 enzyme, but most contain at least a single 30 KDa catalytic domain and one regulatory subunit. PP1 has been found to represent approximately 7% of the phosphatase activity in the human brain. Like the previous two protein phosphatases, PP1 was also found to be capable of dephosphorylating tau *in vitro*, but there have been previous controversies identifying the effect of tau phosphorylation *in vivo* (Gong et al., 2005).

A 2011 study designed a mechanism to test the effect of PP1 dephosphorylation at previously known phosphorylation sites that were linked to tau pathology. Early growth response 1(IGR-1) is a transcription factor that has been previously shown to be significantly upregulated in brain tissue of individuals with AD; IGR-1 was used in this study to stimulate tau pathologies. In doing this, the researchers were able to identify two primary causative factors of the tau phosphorylation from the increased expression of IGR-1. The first factor was the inactivation of PP1 which promoted tau phosphorylation at Ser 396 and Ser 404. However, the study also noted an increase in CDK5 activation, a previously known tau phosphorylator. The study concluded that IGR-1 increases activity of CDK5, which phosphorylates PP1, inhibiting it (Lu, Li, Qureshi, Han, & Paudel, 2011). This study provides evidence to support the claim that PP1 has the capacity to dephosphorylate tau in vivo. However, further research must still be done to

differentiate the degree to which CDK5 activation versus PP1 inhibition individually affects tau phosphorylation.

Furthermore, a 2009 study found yet another interesting link between the Aβ and tau pathology through a PP1 dependent mechanism. The study incubated different levels of Aβ in PC12 cell lines, a cell line that resembles the phenotype of sympathetic ganglion cells. The study found that at 0, 20, and 50 micromolar concentrations of Aβ, there was a notable decrease in the activity of PP1. The ICD-50 for both the fibrillar and oligomeric forms of Aβ was tested on PP1, and it was concluded that Aβ aggregation can increase the inhibitory potency significantly on PP1 (Vintém, Henriques, da Cruz e Silva, Odete A. B, & da Cruz e Silva, Edgar F, 2008). Additionally, the study conducted the same experimental conditions with the previously discussed protein phosphatase, PP2A, and found a similar inhibitory effect, and amplification of inhibition with Aβ aggregation. This lends even more evidence to support the interconnected pathology of Aβ plaques and Neurofibril tangles through PP2A and PP1 pathways.

Discussion

Alzheimer's Disease is a common neurogenerative disease that is common among older populations across the world. When evaluating the many hypotheses regarding the cause and symptomatology of AD, the hypothesis that aligns closest with the current research is the Neurofibril tangles hypothesis. This hypothesis reports that dysfunction among the regulators for tau kinases and phosphatases leads to higher levels of tau phosphorylation in the cell. These hyperphosphorylated tau proteins will begin to preferentially bind to each other, rather than microtubules in the cytoplasm. As hyperphosphorylated tau proteins bind to each other, they begin to build up in the cytoplasm, creating Neurofibril Tangles (Michala Kolarova, Francisco Garcia-Sierra, Ales Bartos, Jan Ricny, & Daniela Ripova, 2012). These Neurofibril Tangles can

eventually lead to cell death and neurological decline which is thought to be a primary cause of the Alzheimer's Disease pathologies seen throughout the scientific literature and clinical studies.

A primary purpose for this review was to analyze the various regulators of tau phosphorylation and evaluate how their dysfunction may lead to Neurofibril Tangles. Regarding the regulation through tau kinases, this review found that increased levels of GSK-3, CDK-5, and AMPK generally have the capacity to increase tau phosphorylation in vitro. However, the mechanisms for increased expression of these kinases vary. GSK-3 has been found to be upregulated by lower levels of activation of an Akt dependent pathway, as well as decreased levels of PKA. CDK-5 was found to be upregulated when there is a high level of conversion from p35 to p25. This results in p25/CDK-5 complexes which increase the phosphorylation of tau. Lastly, AMPK was identified to be upregulated during times of energetic stress. Studies have shown that insulin resistance syndrome causes forms of energetic stress; as a result, tau phosphorylation occurred at higher rates in individuals with insulin resistance syndrome.

The other group of regulators this review examined include three tau phosphatases: PP2A, PP2B, and PP1. It was found that all three of these tau phosphatases generally showed that as their concentration decreased, the levels of tau phosphorylation increased *in vitro*. However, the mechanisms through which this occurs varied depending on the phosphatase. Scientists believe that decreased levels of PP2A could be, in part, due to the ApoE 4 allele. Researchers have discovered that if an individual has this allele, they will have decreased levels of functional PP2A in nervous tissue. PP2B has been shown to be inversely correlated with the activation levels of DSCRBI *in vitro*. Lastly, in the presence of higher levels of IGF-1, researchers have reported a decrease in the level of PP1 activity *in vitro*.

Given the complex, interconnected nature of biology, these studies do not include all the relationships between the previously mentioned tau kinases and phosphatases. However, this review hopes to clearly demonstrate some of the proposed mechanisms and organize how the various tau regulators may be related. Furthermore, the mechanisms above describe the direct causes resulting in dysfunction of these tau regulators; it does not evaluate how different tau regulators may also play a regulatory role in each other's activity.

Synergistic and Antagonist Effects Between Tau Regulators

Understanding the effects that tau kinases and phosphatases have on each other during regulation of tau phosphorylation will be important as researchers look to find effective methodologies to treat and prevent AD symptomatology. One interesting finding noted in this study was the conflicting effects that were observed through the decreased activation of PP2A in relation to GSK-3β activity. PP2A has previously been observed to decrease tau phosphorylation in many studies when applied to cell cultures and brain tissues. However, more recent studies have identified that GSK-3β activation is upregulated in the presence of PP2A because it dephosphorylates the Serine 9 residue, which enhances its activity. Therefore, although PP2A has been proven to dephosphorylate tau, it also dephosphorylates the tau phosphorylator, GSK-3β, which has been shown to increase tau phosphorylation. Similar findings have also been observed with AMPK; in other words, PP2A has also been shown to activate tau kinases, which increase the rate of phosphorylation of tau. Thus, more studies must be designed to further evaluate the mechanistic and clinic applications these findings have on PP2A research with respect to tau hyperphosphorylation.

Another interesting finding from this review was the synergistic effect of CDK5 and PP1 on tau hyperphosphorylation. Previous studies have found that increasing activation of CDK-5

causes it to more readily phosphorylate Tau *in vivo*. However, more recent studies analyzing the effect CDK-5 has on other tau regulators has found that increasing CDK-5 has the capacity to phosphorylate PP1. Phosphorylation of PP1 inhibits its capacity to remove phosphate groups from tau, so it cannot reverse the effects of other tau kinases like CDK-5. Therefore, studying CDK5 in future experiments may be a more tactical research approach, as it has the capacity to hyperphosphorylate tau and to inactivate PP1, which further amplifies tau hyperphosphorylation.

One last interesting concept was reported on the regulation of GSK-3 *in vivo*. PKA was found to have the capacity to directly phosphorylate GSK-3, inhibiting it. Researchers also found a separate pathway through which GSK-3 can have its activity inhibited. In increased levels of IGF-1, an Akt dependent pathway was found to be downregulated due to the change in energy requirements of the cell. As this pathway is downregulated, the activity of GSK-3 is upregulated, which can potentially contribute to the phosphorylation for tau. Furthermore, it was found that these two pathways—PKA and Akt— may synergistically coregulate the activity of GSK-3 depending on the context of environmental energy levels. However, further research is still needed to evaluate how these two seemingly different pathways may interact to regulate the activity of GSK-3, and how dysfunction of this interaction could cause hyperactivity of GSK-3.

Connections Between Amyloid Beta and Tau Pathologies

Understanding the significant links between NFTs and Aβ Plaques is essential when evaluating AD pathology. Furthermore, a 2002 study investigated the interdependence between these two pathways by using hippocampal neurons from tau knockout mice and testing them for their vulnerability to Aβ mediated cell death. The study found that in these mice without functional tau, it was resistant to cell death via Aβ pathways (Rapoport, Dawson, Binder, Vitek,

& Ferreira, 2002). This study demonstrates the need to evaluate these pathways together, given the intertwined dependences of their pathologies.

Throughout this research review, there were four identified pathways that link the pathological experimental findings between tau and Aβ. The first mechanistic link between the pathology of these two proteins is through an Akt mediated pathway. Aβ was found to decrease Akt activation; this reduces inhibition of GSK-3 via phosphorylation. Therefore, GSK-3 will be upregulated, and other studies have shown that higher levels of GSK-3 increase hyperphosphorylation of tau.

The second mechanism that was identified to relate Aβ and Tau pathologies involve a CDK-5 dependent pathway. Given that p25/CDK complex phosphorylates tau to a much greater degree than the p35/CDK complex, this will cause an increase of NFTs in the cell cytoplasm. Furthermore, researchers have found that when there are higher levels of P25/CDK5 complexes in the cell, there is also an increased rate of production of Aβ precursors.

A third mechanism that was shown to link Aβ to tau was through an AMPK dependent pathway. Increased levels of Aβ were found in individuals with insulin resistance syndrome. Furthermore, insulin resistance syndrome was found to upregulate the energy monitoring protein, AMPK, in the cell. AMPK has been proven to increase phosphorylation of tau and was found at higher levels in AD brain tissue; therefore, it presents a third possible link between Aβ and tau.

The fourth mechanism that was reviewed in this study that links Aβ to tau is a PP1 dependent process. Increasing levels of Aβ was found to be a direct inhibitor of PP1. This effect is even more amplified as the Aβ begins to clump together as seen in Aβ Plaque pathology. This inhibition of PP1 decreased the dephosphorylation of tau, which can lead to clumping and

eventually to Neurofibril Tangles. This illustrates a fourth link between the pathological significance of Aβ Plaques and NFTs.

These mechanisms illustrate the intertwined nature that Aβ Plaques and NFTs have in relation to Alzheimer's Disease pathology. Given the previous mechanisms, Aβ seems to directly, and indirectly, impact many regulators of tau kinases and tau phosphatases. By working to regulate upstream factors like Aβ, researchers could more efficiently target the original causes of dysfunction rather than treating the abundant downstream effects of the original dysfunction. In conclusion, this information further supports the drive to not only study NFTs, but also study other pathological mechanisms to AD, specifically the Aβ Plaques hypothesis.

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