Role of WWOX/WOX1 in Alzheimer's disease pathology and in cell death signaling

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1. ABSTRACT

Alzheimer's disease (AD) is the most common form of dementia with a progressive course. AD pathology is a manifestation of the underlying severity and neuroanatomic involvement of specific vulnerable brain regions and circuits that are responsible for neuronal dysfunction and death. The etiology of AD is largely unknown. It has been hypothesized that multiple factors, including genetic components, oxidative stress, intracellular or extracellular accumulation of amyloid, dysfunction of cystoskeletal and synapse components, neuronal loss by apoptosis, neuronal excitotoxicity, inflammation, mitochondria dysfunction, etc., may play important roles in the onset of the disease. WWOX/WOX1 is a candidate tumor suppressor. Human *WWOX* gene, encoding the WW domain-containing oxidoreductase (designated WWOX, FOR, or WOX1) protein, has been mapped to a fragile site on the chromosome ch16q23.3-24.1. Functionally, the WW domain is not only a tumor suppressor, but also a participant in molecular interactions, signaling, and apoptosis in many diseases. In this article, we review the potential mechanism by which WWOX/WOX1 may participate in the pathogenesis of AD with a focus on cell death signaling pathways in neurons.

2. INTRODUCTION

Alzheimer's disease (AD) is the most common cause of dementia in elderly populations, presenting as a progressive disorder with clinical, biological, and pathological features occurring along the entire cognitive spectrum from normal to end-stage disease (1). Aging is the best correlative risk factor for AD (2). The disease afflicts one in eight people age older than 65 and nearly one in two people over 85. The cause of AD is unknown. Aging is the biggest driving force behind Alzheimer's; some of the same factors that trigger heart disease—obesity, high cholesterol, and diabetes—also increase the risk of dementia. AD is pathologically characterized by the presence of extracellular senile plaques, which consist of a core of amyloid-beta peptide and intracellular neurofibrillary tangles (NFTs), as well as the selective loss of neurons and synaptic connections (3-6). The exact cause(s) that induce(s) these pathological characteristics are poorly understood. AD pathology has been hypothesized as being the result of 1) genetic mutation; 2) oxidative stress in neurons; 3) coupling signal transduction pathways of amyloid beta receptors; and 4) hyperphosphorylation of Tau, a microtubule associated protein (7-8). The pathological characteristics of AD are not independent events. The accumulation of oligomeric and fibrillar

amyloid beta in neurons may induce a series of neuronal signal transduction events to promote Tau hyperphosphorylation (8-12). The hyperphosphorylated Tau loses the ability to bind to microtubules, which leads to the loss of neurons. It has been suggested that soluble phospho-Tau, but not aggregated Tau, is the primary factor for cellular toxicity. Aggregated Tau/NFTs containing neurons show prolonged longevity, suggesting the hypothesis of a neuroprotective role for NFTs (13, 14).

WW domain-containing oxidoreductase (designated WWOX, FOR, or WOX1) is a candidate tumor suppressor. Human *WWOX* gene, encoding the WWOX/WOX1 protein, has been mapped to a fragile site on the chromosome ch16q23.3-24.1 (15-17). Loss of heterozygosity of this chromosomal region has been demonstrated in many types of cancers. The *FHIT* gene is also located on a chromosomal fragile site (18). The WWOX/WOX1 gene encodes a 414 amino acid protein of 46.6 kDa molecular weight (15-17). WWOX/WOX1 protein possesses a nuclear localization sequence, two *N*-terminal WW domains (containing conserved tryptophan residues) and a *C*-terminal short chain alcohol dehydrogenase/reductase (ADH/SDR) domain (15-17). WW domains have been shown to interact with a wide variety of signaling proteins and functioning as adaptor proteins, transcriptional co-activators, and ubiquitin ligases. Via its first WW domain, WWOX/WOX1 is able to associate with and modulate the functions of a spectrum of proline-rich ligand containing proteins, such as p53, p73, Erb-4, c-Jun N-terminal kinase (JNK), c-Jun, runt-related transcription factor 2, dishevelled homolog protein-2 (Dvl-2), ezrin, etc (19-27). However, upregulation of the proapoptotic p53 is independent of the WW domain and proline-rich ligand motifs (28). WWOX/WOX1 is more than just a tumor suppressor. WWOX/WOX1 enhances tumor necrosis factor (TNF) cytotoxicity by down-regulation of the apoptosis inhibitors, Bcl-2 and Bcl-xL. Overexpression of the full-length WWOX/WOX1 or just its WW domain region induces apoptosis (17, 22, 28). Under stress conditions, WWOX/WOX1 can be activated at Tyr33 by phosphorylation of this tyrosine residue; the activated WWOX/WOX1 binds to p53 and co-translocates to the mitochondria or to the nucleus (22). Reducing the expression levels of WWOX/WOX1 by siRNA abolishes ultraviolet (UV) light-induced p53 activation and cell death (19). Inhibition of murine double minute 2 (MDM2) increases WWOX/WOX1 binding and stability of p53 (19). JNK1, a mitogen-activated protein kinase (MAPK) involved in stress response and apoptosis (29-30), physically interacts with WWOX/WOX1 and inhibits WWOX/ WOX1-mediated apoptosis (22).

WWOX/WOX1 may modulate the development of AD. In the brains of AD patients, WWOX/WOX1 phosphorylation at Tyr33 is significantly downregulated in the hippocampal neurons, but Tau phosphorylation and glycogen synthase kinase-3 (GSK-3) activation are elevated when compared to age-matched controls (32). These findings suggest that downregulation of WWOX/WOX1 in AD is essential to induce Tau hyperphosphorylation and subsequent generation of NFTs. Furthermore,

WWOX/WOX1 inhibits Wnt/beta-catenin pathway through sequestration of Disheveled (Dvl) protein (26). It has been shown that loss of Wnt signaling components determine the onset and development of AD (31). WWOX/WOX1 is involved in apoptotic and stress responses *in vivo* and *in vitro* (17, 33-35), regulation of gene transcription (33-35), and neural development, injury, and degeneration (e.g. Alzheimer's disease) (32, 43-47). There is limited literature discussing the role of WWOX/WOX1 in AD. However, given the known functions of WWOX/WOX1, it is likely that WWOX/WOX1 may participate in the pathogenesis of AD through direct or indirect interaction with p53, JNK1, Wnt/beta-catenin, Tau, GSK3-beta, amyloid beta, cAMP response element-binding (CREB), Transforming growth factor-beta (TGF-beta), and/or other potential protein partners to modulate or regulate neural functions (19, 22, 26, 32, 46, 48, 49).

3. WW DOMAIN-CONTAINING PROTEINS

WW domains are 38-40 amino acid residue units that fold into a three-stranded beta-sheet structure. A flat binding surface for the proline-rich ligand is formed by conserved hydrophobic residues. The domain name is derived from two conserved Tryptophan residues spaced 20 to 22 residues apart within the consensus sequence (50-53). The functions of WW domains derive from recognition of proline-rich peptide motifs and phosphorylated serine/threonine-proline sites (52-54, 57). WWOX/WOX1 and its binding partners are involved in many molecular processes, such as transcription, RNA processing, and cytoskeletal regulation, etc (54, 55). The WW domains can be arranged in tandem repeats at various distances in a single protein and function in a synergistic or an independent manner (56). A schematic diagram of WWOX/WOX1 protein is shown in Figure 1. Tyr33 phosphorylation in WWOX/WOX1 occurs when cells are exposed to TNF-alpha, TGF-beta, staurosporine, etoposide, UV irradiation, complement C1q, and sex hormones including estrogen and androgen (19, 22, 35, 49, 58, 59). Downregulation of activated CDC42 kinase 1 by neuronal precursor cell-expressed developmentally downregulated 4-2, E3 ubiquitin ligase (NEDD4-2) prolongs the lifetime of WWOX/WOX1 (60, 61). MDM2, an E3 ubiquitin ligase, is regulated by binding to WWOX, and is involved in central nervous system degeneration (19, 62). Peptidylprolyl cis/trans isomerase, NIMA-interacting 1 (Pin1), containing only a WW domain (63), is involved in both cancer and Alzheimer's disease (64, 65). Pin1 is upregulated in cancers but downregulated in Alzheimer's disease, and participates in immune regulation (66).

3.1. Tauopathy and WWOX/WOX1 in AD

Tau is a microtubule-associated protein functioning to promote microtubule assembly and stabilize microtubules (67). Microtubules are essential for the axonal transport of neurons (67). Phosphorylation of Tau affects axonal flow and cell viability in mature and developing neurons (67). There are more than ten serine/threonine protein kinases that have been shown to phosphorylate Tau *in vitro*. According to their motif-specificities, these
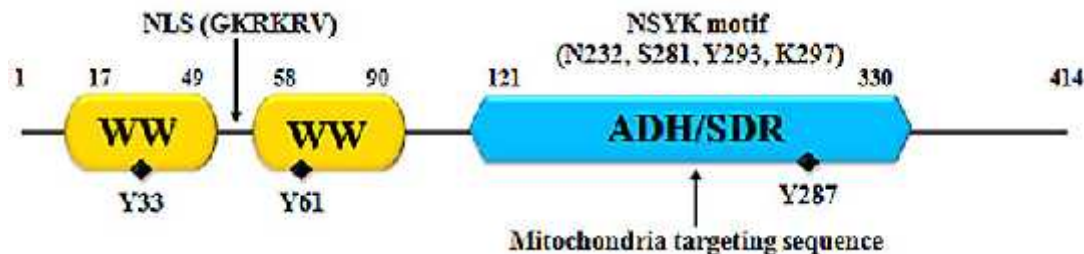
Figure 1. Human WWOX/WOX1 protein structure. Human WWOX/WOX1 gene encodes a 414 amino-acid-long chain protein with a molecular weight of 46.6 kDa. WWOX/WOX1 possesses two N-terminal WW domains, a nuclear localization signal sequence (NLS), and a C-terminal short-chain alcohol dehydrogenase (ADH/SDR) domain. There are three tyrosine phosphorylation sites. Tyr33 phosphorylation activates WWOX/WOX1. The potential function of Tyr61 and Tyr287 phosphorylation remains elusive. The conserved catalytic tetrad N-S-Y-K motif is shown (N232, S281, Y293, and K297). A mitochondrial targeting sequence in WWOX/WOX1 is mapped within the ADH/SDR domain (amino acid sequence 209–273).

kinases can be divided into proline-directed protein kinases and nonproline-directed protein kinases (68-73). GSK3-beta, a proline-directed kinase, is most frequently implicated in the abnormal hyperphosphorylation of Tau in AD brain. Tau gene mutations, aberrant mRNA splicing, and abnormal posttranslational modifications, have been found in a number of neurodegenerative disorders including Alzheimer's disease, frontotemporal dementia, Pick's disease, cortical basal degeneration, progressive supranuclear palsy (74).

### 3.2. Enzymes participating in WWOX/WOX1-mediated AD pathology

GSK3-beta participates in both Tau and amyloidal pathologies in AD (77). Overexpression of GSK3-beta results in Tau hyperphosphorylation and neurodegeneration in conditional GSK3-beta transgenic mice (78). Upregulation of GSK3-beta inhibits long-term potentiation with synapse-associated impairments *in vitro* (79). Beta amyloid activates GSK-3 through the inhibition of protein kinase C (PKC) activity (80). Peroxynitrite induces Alzheimer-like Tau modifications and accumulation in rat brain via GSK-3 activation (81). Synergistic effects of protein kinase A (PKA), cyclin-dependent kinase 5 (CDK5), dual-specificity tyrosine phosphorylation-regulated kinase1A with GSK-3, and reciprocal functions of GSK-3 with protein phosphatase-2A (PP-2A), and protein phosphatase 5 (PP5) on Tau hyperphosphorylation have been proposed (82). GSK3-beta, JNK1, CDK5, extracellular signal-regulated kinase (ERK), and several others are known to hyperphosphorylate Tau *in vivo* (32). In comparison with age-matched normal controls, we found that WWOX/WOX1, its isoform WOX2, and their Tyr33-phosphorylated forms are significantly downregulated, along with increased Tau hyperphosphorylation, in the AD hippocampal neurons (32). When WWOX/WOX1 expression was knocked down by siRNA in SK-N-SH cells, endogenous Tau in these cells became phosphorylated selectively at Thr212/Thr231 and Ser515/Ser516 along with enhanced phosphorylation of GSK3-beta (at Tyr 216) and ERK, and NFT formation. Phosphorylation of these residues can be found in the PHF-Tau and NFTs from clinical specimens (32).

WWOX/WOX1 interacts with Tau, JNK1 and GSK3-beta in the extracts of rat brains and cultured cells (32). Mapping analysis showed that WWOX/WOX1 binds Tau via its C-terminal ADH /SDR domain.

Heat shock proteins (HSPs) target and direct the non-repairable misfolded protein aggregates to the ubiquitin proteasomal pathway (75). The E2 enzyme UbcH5B and C-terminus of the Hsc70-interacting protein (CHIP)-Hsc70 complex, also called Tau E3 ligase, ubiquitinized phosphorylated Tau extracted from AD brains *in vitro*. The ubiquitinated PHF-Tau is not degraded but deposited in the NFTs in AD brain (76). Overexpression of CHIP in AD promotes degradation of the hyperphosphorylated Tau in rat brain and neuroblastoma N2a cells (77).

3.3. Tau phosphorylation and WWOX/WOX1 in neuron survival

There has been controversy about the toxicity of Tau polymerization (82, 83). Aggregated Tau is toxic to cells (84, 85). The polymerized Tau proteins *in vitro* lose the biological activities in promoting microtubule assembly and binding to the microtubules (84). On the other hand, there are reports showing that polymerization of Tau is not relevant to cell toxicity (82). In mice that express mutant human Tau$_{P301L}$, a prominent neuronal loss is shown in the hippocampal CA1 region. Suppression of mutant Tau expression prevents further neuronal loss without reducing neurofibrillary pathology (86). The dissociation of neuronal loss and accumulation of neurofibrillary pathology imply that formation of NFTs may not necessarily lead to neuronal death. By quantitative analysis of neuronal loss and NFT formation for an estimation of disease duration, the presence of Tau filaments did not correlate directly with the death of individual neurons, suggesting that NFT may not be obligatory for death of CA1 hippocampal neurons in AD patients (87-89). Tau phosphorylation is upregulated during oxidative stress by lipid peroxidation products (90). Acute oxidative stress and mild heat stress induce the accumulation of dephosphorylated Tau in neuronal nuclei. Increased interaction of endogenous Tau with neuronal genomic DNA prevents heat stress-induced damage (91). Therefore, the regulation of Tau phosphorylation in adult
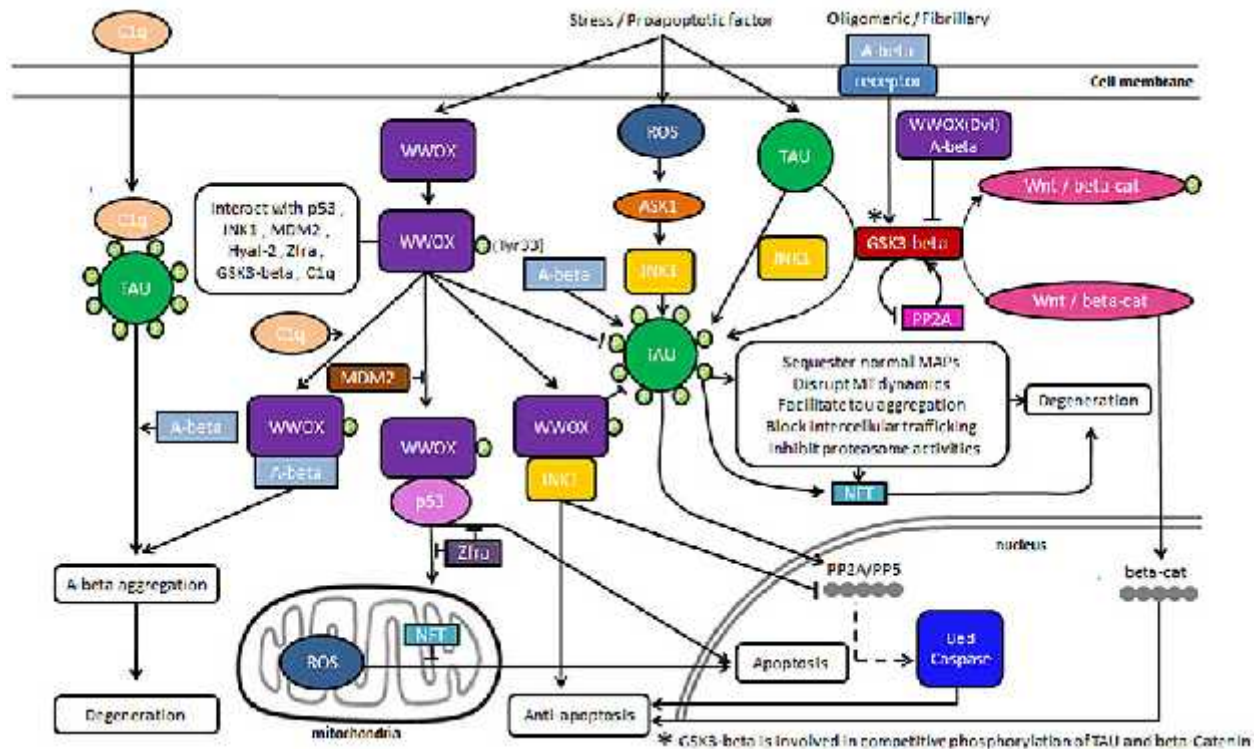
Figure 2. WWOX/WOX1 participates in regulating AD pathology. Stress or proapoptotic factor stimulation results in increased WWOX/WOX1, ROS, and Tau expression in the neuron. WWOX/WOX1 is phosphorylated at tyrosine 33 which interacts with p53, JNK1, GSK3-beta, Zfra, MDM2, and C1q. Re-localized phosphorylated WWOX/WOX1 at tyrosine 33 to the nucleus can induce apoptosis. WWOX/WOX1 works synergistically with p53 and re-localizes to the mitochondria or nucleus to promote apoptosis, which can be blocked by Zfra and MDM2. JNK1 physically interacts with phosphorylated WWOX/WOX1 to block WWOX/WOX1-induced apoptosis. WWOX/WOX1also interacts with JNK1 and GSK3-beta to prevent Tau phosphorylation. C1q binds to phosphorylated Tau and amyloid beta. C1q/WOX1 signals result in the formation of WWOX/WOX1 and amyloid beta-containing fragments, which enhance amyloid aggregation and neurodegeneration. ROS promotes Tau hyperphosphorylation through ASK1 and JNK signaling. ROS alone also causes cell apoptosis. Amyloid beta, via GSK3-beta, induces Tau hyperphosphorylation. GSK3-beta is also involved in competitive phosphorylation of Tau and beta-catenin. WWOX/WOX1 partners with Wnt/beta-catenin through sequestration of Dvl proteins to inhibit GSK3-beta-mediated beta-catenin phosphorylation. Translocation of beta-catenin to the nucleus inhibits apoptosis. Hyperphosphorylated Tau results in NFT containing "sick neuron", and subsequent neurodegeneration. Hyperphosphorylated Tau activates PP2A or PP5, and through indirect inhibition of BCL2-antagonist of cell death (Bad) and caspase transcription in the nucleus to protect the neuron from apoptosis.

mammalian brain may represent a naturally-occurring process that is associated with neuroprotective mechanisms (92, 93, 95).

Beta-catenin is a major component involved in Wnt signaling. Beta-catenin can be phosphorylated by GSK3-beta for creating a signal for rapid degradation via proteosomal pathway. The exact connection between Tau and beta-catenin phosphorylation in not known. It has been suggested that Tau and beta-catenin compete for GSK3-beta-mediated phosphorylation (67). Increased Tau phosphorylation inhibits beta-catenin phosphorylation, thus facilitating the function of beta-catenin in preventing apoptosis (67) (Figure 2).

In AD brain, the mechanism for connecting degenerated neurons with spatial temporal-specific patterns of Tau hyperphosphorylation into cell death signaling remains to be established. WWOX/WOX1 may, through its regulation with Tau, GSK3-beta, JNK, Wnt/beta-catenin, stress-induced ROS, C1q, etc., participate in these signaling events in AD brains (32, 36, 59, 67, 90). WWOX/WOX1 binds directly to Tau through its ADH/SDR domain as evident by yeast two-hybrid analysis (32). Silencing of WWOX/WOX1 by siRNA increases the binding of Tau to GSK3-beta and phosphorylation of Tau, indicating that WWOX/WOX1 may be involved in regulating GSK3-beta activity in cells (32). It has been demonstrated that inhibition of GSK3-beta plays an essential role in neuronal differentiation (94). Overexpression of WWOX/WOX1 enhances the SH-SY-5Y cell differentiation with or without the treatment of retinoic acid (RA). RNAi-mediated knockdown of WWOX/WOX1 in RA-differentiated SH-SY-5Y cells caused a decrease in neurite outgrowth (unpublished data).

Activation of GSK3-beta leads to AD-like memory deficit and neuronal dysfunction and death similar to AD (96-98). Inhibition of GSK3-beta activity by lithium salts protects against neuronal degeneration and death induced by amyloid beta and Tau hyperphosphorylation *in vitro* and *in vivo* (96-99). Inactivation of GSK by Wnt signaling prevents Tau phosphorylation in the GSK-dependent epitopes (96, 100). GSK3-beta maintains a hyperactive state in AD, which leads to the hyperphosphorylation of Tau. GSK3-beta regulates amyloid precursor protein (APP) metabolism and overproduction of amyloid beta, which results in reduced neurogenesis and increased apoptosis (7). A neuronal protective hormone, 17-beta-estradiol increases the binding of WWOX/WOX1 and GSK-3 with Tau (32). Serine and threonine are function-associated phosphorylation sites of Tau (82). Given that WW domains functions derive from recognition of proline-rich peptide motifs and phosphorylated serine/threonine-proline sites, detailed dissections of how WWOX/WOX1 is involved in modulate and regulate Tau phosphorylation and NFT formation at specific serine/threonine sites might shed light on how to prevent neurodegeneration.

3.4. Potential key linkers for Alzheimer's disease pathology and WWOX/WOX1

Tau is essential to amyloid beta-induced neurotoxicity (101). Amyloid beta causes degeneration in Tau expressing neurons, but not in Tau-depleted neurons (102). Overloading amyloid beta induces hyperphosphorylated Tau in AD brains directly or indirectly. GSK3-beta is a key linker to the accumulation of amyloid beta and hyperphosphorylation of Tau (8). In a cell-free model *in vitro*, hyperphosphorylation of Tau by GSK3-beta is sufficient to cause tangle-like aggregates which are similar to NFT isolated from AD brains (103). Transgenic mice overexpressing GSK-3beta induces Tau hyperphosphorylation and tangle-like filaments formation in hippocampal neurons as well as atrophy of the hippocampal dentate gyrus (104). Active GSK3-beta levels in cortical neurons are increased at different stages of neurofibrillary degeneration (105). Phosphorylation of Tau at Thr231 enhances subsequent hyperphosphorylation of Tau by GSK3-beta (80,106). Tau phosphorylation is also regulated by the interactions between WWOX/WOX1, GSK3-beta, JNK, and Wnt/beta-catenin (32, 36, 67, 90). Tau phosphorylation is inhibited by the interaction between WWOX/WOX1, JNK1 and GSK3-beta (Figure 2). The physical interactions between WWOX/WOX1, Tau, JNK1 and GSK3-beta have been demonstrated in the extracts of rat brains and cultured cells (32). These findings suggest that WWOX/WOX1 might participate in AD pathology through its protein binding partners. Figure 2 summarizes how WWOX/WOX1 interacts with its potential domain-binding partners to participate in AD pathology.

3.5. ROS and WWOX/WOX1 in AD

Reactive oxygen species (ROS) generated by beta amyloid disrupt mitochondrial respiration (107-109). Mitochondrial damage promotes neurodegeneration and cell death (109). The activation of apoptosis signal-regulating kinase 1 (ASK1) induces the activation of JNK and p38

MAPK. Amyloid beta induces neuronal cell death through ROS-mediated ASK1 activation (110). The c-Jun kinase is a downstream effector of JNK and is associated with the activation of caspase-3 and neuronal apoptosis. The activation of JNK and p38 MAPK in AD-induced Tau phosphorylation generates Tau protein pathology but is not coupled with neuronal apoptosis through the c-Jun kinase pathway (111, 112). How to explain this dissociation between neuronal loss and accumulation of neurofibrillary pathology in AD remains elusive. Mouse WWOX/WOX1 interacts with phosphorylated c-Jun in the sciatic axotomy neuronal injury in rat, which enhances the promoter activation governed by c-Jun (46). On the other hand, WWOX/WOX1 inhibits apoptosis to protect neurons in a time-lapsed chronicled fashion in dorsal root ganglia of rat (46). Whether this phenomenon would occur in cortical neuron, and JNK1 and WWOX/WOX1 interaction would induce oxidative stress and free radical is unknown.

3.6. Neuronal cell death signaling and WWOX/WOX1 in AD

Neuronal apoptosis occurs in AD brain. The apoptotic DNA fragmentation and expression of apoptotic signaling proteins are observed in the neocortices and hippocampi of postmortem AD brains (113). Amyloid beta alone induces neuronal apoptosis *in vitro* (114). The expression of TNF receptor I (TNFR1), a death receptor, is increased in AD and is related to the apoptotic process through its ligand, TNF-alpha (115). TNF-alpha induces apoptosis through activation of Fas-associated protein with death domain (FADD), JNK or nuclear factor-kappaB (NF-κB). JNK is activated in AD patients and mouse models of AD, which is associated with expression of pro-apoptotic genes and activation of caspases-3 and caspase-9 (118). WWOX/WOX1 increases the cytotoxic function of TNF in killing cancer cells by interacting with TNFR1-associated death domain (TRADD)/FADD and TRAF2 (13, 28). TNFR1/FADD recruits TRAF2 that leads to NF-κB activation. In AD, amyloid beta peptide activates NF-κB and induces apoptosis (116, 117). Amyloid beta peptides promote pathological Tau filament assembly and NFTs formation in neurons (119). We found that rapid activation of JNK1 and WWOX/WOX1 during the acute phase of injury is critical for neuronal survival or death, however, chronic and concurrent activation of WOX1, CREB, and NF-κB occurs in small neurons just prior to apoptosis (46). WWOX/WOX1 inhibits pro-survival CREB-, CRE-, and activator protein 1(AP-1)-mediated promoter activation *in vitro*. In contrast, WWOX/WOX1 enhances promoter activation governed by c-Jun, Elk-1(Ets Like gene1) and NF-κB (46). WWOX/WOX1 directly activates NF-κB-regulated promoter via its WW domains (46). Zfra (31-amino-acid zinc finger-like protein) interacts with death domain protein TRADD to regulate TNF-mediated cell death, and downregulates NF-kappaB, JNK1, p53 and WWOX/WOX1 during stress response (23). Zfra interferes with WWOX/WOX1 and p53-induced apoptosis by blocking its translocation to the mitochondria or nucleus (23).

WWOX/WOX1 is a proapoptotic protein, capable of interacting with p53 (19, 28, 33). p53
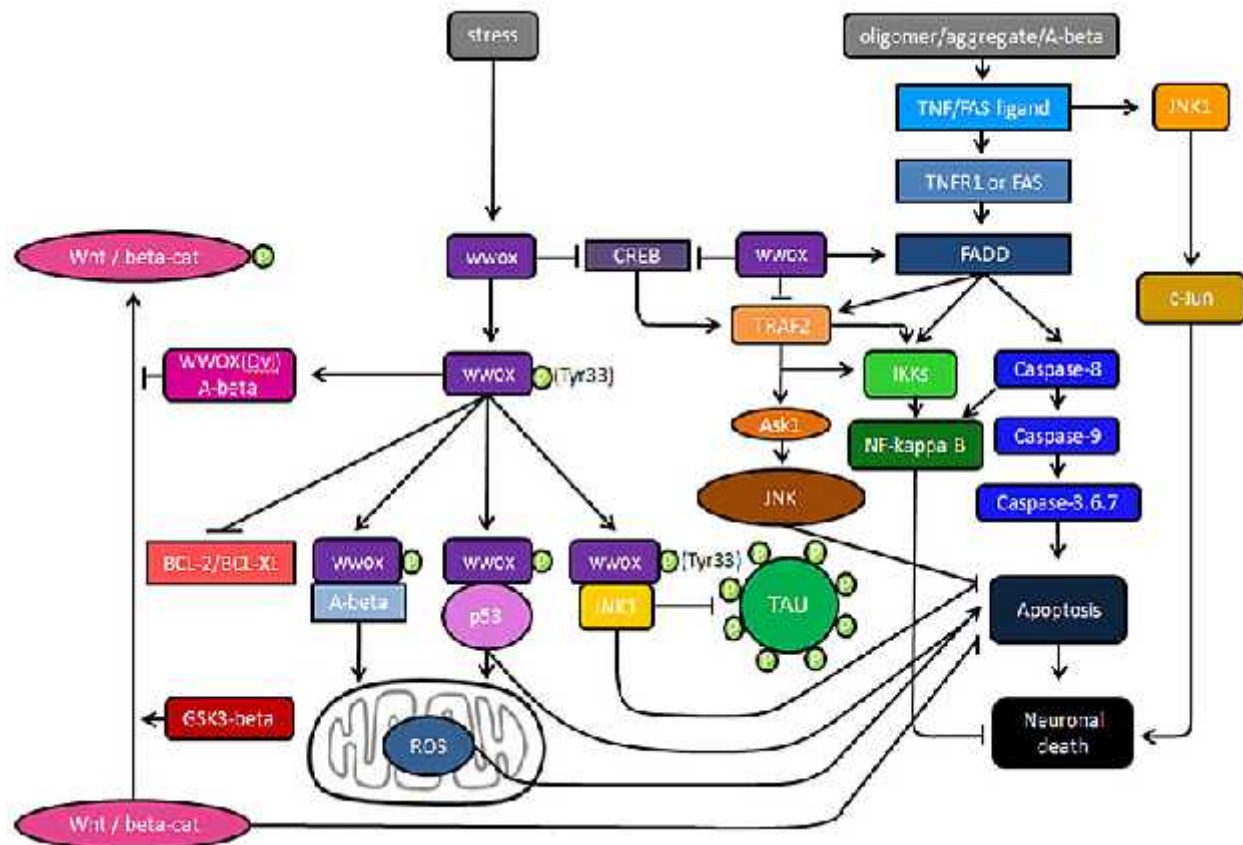
Figure 3. WWOX/WOX1 participates in cell death signaling in Alzheimer's disease. Oligomeric/fibrillary amyloid beta aggregates are lethal to neurons through TNF/FAS ligand or JNK1 signaling pathways. WWOX/WOX1 enhances TNF apoptosis by interacting with FADD and TRAF2. TNF binds to the membrane p55 TNFR1, which recruits FADD and activates caspase 8, which in turn activates caspase 9, caspase 3 and downstream caspase 6 and 7. TNFR1/FADD recruits TRAF2 that leads to NF-κB activation. In a second link, TRAF2 activates apoptosis signal-regulating kinase 1 (ASK1), which in turn activates JNK. WWOX/WOX1 blocks CREB and TRAF-2, which is followed by activation of NF-κB and JNK to inhibit apoptosis. The exact role of WWOX/WOX1 and these proteins' interactions in TNF signaling to balance the cell's fate between survival and death remains elusive. Phosphorylated WWOX/WOX1 at tyrosine 33 (pWOX1) either by translocation to the mitochondria or the nucleus to work directly or indirectly with amyloid beta or p53 to enhance apoptosis. pWOX1 interacts with JNK1 or GSK3-beta to inhibit Tau phosphorylation, which can either be protective or harmful to neurons in AD depending on the chronicle (acute or chronic) of Tau phosphorylation. WWOX/WOX1 also partners with the Wnt/beta-catenin through sequestration of Dvl proteins to inhibit GSK3-beta-mediated beta-catenin phosphorylation, which inhibits apoptosis.

transcriptional activity depends on posttranslational modifications and protein-protein interaction. Homeodomain interacting protein kinase 2 (HIPK2) is an evolutionary conserved serine/threonine kinase to maintain wild-type p53 function (122). Soluble beta amyloid peptides is involved in HIPK2 degradation, which results in misfolded p53 and altered vulnerability of cells to a noxious stimulus, suggesting that conformationally changed p53 can be a putative biomarker for AD (120, 121, 122). Given that WWOX/WOX1 and p53 induce apoptosis synergistically, whether or not WWOX/WOX1 interacts with amyloid beta peptides or HIPK2 remains to be established. It is important to discerning the link between transient overexpression of WWOX/WOX1 ADH/SDR domain and accumulation of amyloid beta peptide, Tau phosphorylation, and formation of NFT. Also to determine how WWOX/WOX1 interacts with critical signaling

proteins such as TNF-alpha, p53, JNK1, NF-κB, Wnt/beta-catenin, Pin1, CREB, etc., to regulate cell death *in vitro* and *in vivo* might be the focus of future research to understand the role of WWOX/WOX1 in neurodegenerative cell death in AD. It is not clear whether or not WWOX/WOX1 participates in necrotic and autophagic cell death in AD. Figure 3 summarizes the potential role(s) of WWOX/WOX1 in cell death signaling in AD.

3.7. WWOX/WOX1 and inflammation in AD

C1q, a subcomponent of C1, is capable of binding to amyloid beta in the beta-sheet conformation and activate complement *in vitro* (123). Absence of C1q leads to less neuropathology in transgenic mouse models of AD (124). On the other hand, C1q protects early stages of neuronal injury by rapid clearance of apoptotic cells and/or cellular debris to prevent inflammation (125). Tenner *et al.*

reported that the contribution of complement activation pathways to neuropathology differs among mouse models of AD (124). The alternative complement pathway activation of a C3-independent cleavage of C5 determines the progression of neuropathology in 3xTg mice versus other murine models (124). C1q binds to hyperphosphorylated Tau and activated complement *in vitro* (126, 127). In Down's syndrome with AD, compaction of amyloid beta 42 deposits activates the classical complement cascade, which progresses to make neuronal expression of the membrane attack complex (MAC) as a response to amyloid beta plaque maturation (128). C1q binds to Tau and amyloid beta and is a potent facilitator of amyloid beta aggregation (123, 126, 128). C1q is capable to activating WWOX/WOX1 bypassing the classical pathway activation (59). When overexpressed, WWOX/WOX1 signals with C1q to induce apoptosis, which is followed by fragmentation of WWOX/WOX1-containing microvilli clusters formed in between prostate DU145 cells (59). C1q alone signals WWOX/WOX1 activation for apoptosis. This event occurs only when cells have sufficient amounts of intracellular WWOX/WOX1 (59). The underlying mechanism of how WWOX/WOX1 interacts with C1q in AD pathology and cell death signaling remains to be determined.

3.8. TGF-beta1 and WWOX/WOX1 in AD

TGF-beta1 is crucial in regulating neuroprotection and neurodegeneration (132). Long-term overexpression of TGF-beta1 in mice causes neurodegeneration (132). Many WW domain-containing proteins participate in the TGF-beta signaling (35). TGF-beta1 binds cell surface hyaluronidase Hyal-2 in in type II TGF-beta receptor-deficient cells, followed by recruiting WWOX and mothers against decapentaplegic homolog 4 (Smad4) to regulate gene transcription, growth and death (49). Recently, we have demonstrated that a small TGF-beta1-induced antiapoptotic factor (TIAF1) is involved in the pathogenesis of AD (129). TGF-beta1 and environmental stress induces TIAF1 self aggregation in a type II TGF-beta receptor-independent manner in cells (129). Smad4 interrupts the aggregation formation (129). Aggregated TIAF1 induces apoptosis in a caspase-dependent manner. By filter retardation assay, TIAF1 aggregates were found in the hippocampi of non-demented humans and AD patients (129). TGF-beta1 signals the binding of TIAF1 with Smad4 to form a complex, which relocates to the nuclei to modulate gene transcription. Smad4 is critical for supporting the generation of membrane APP via regulation of gene transcription (130, 131). Furthermore, TIAF1 binds and stabilizes membrane APP. TGF-beta1 induces TIAF1 aggregation and reduces its binding with APP, and causes APP dephosphorylation (129). Dephosphorylated APP undergoes enzymatic cleavage and subsequent production of amyloid beta monomer, intracellular domain of the APP intracellular domain (AICD), and amyloid fibrils (133). Thus, a balanced state of phosphorylation of APP is critical in determining plaque formation. Overexpressed Smad4 alone induces apoptosis of cancer and neuronal cells. It seems that TGF-beta-induced TIAF1 aggregation and Smad4-mediated APP gene activation occur concurrently (129).

TIAF1 aggregates bind to Smad4 to prevent nuclear relocation (129). We suggest that TIAF1 aggregation occurs preceding generation of amyloid beta and amyloid fibrils, and the TIAF1/amyloid fibril aggregates facilitate plaque formation (129). TGF-beta1 exerts neuroprotective or degenerative function depends on the duration of its exposure in transgenic mice (132). Long-term TGF-beta1 exposure results in irreversible amyloid fibrils and apolipoprotein E (ApoE) depositions, even after silencing of the transgene or TGF-beta1 removal (132). WOX1/WWOX and TIAF1 both participate in regulating the activation of Smad-driven promoter via the type II TGF-beta receptor-independent manner to induce apoptosis or neurodegeneration (49, 129). This different outcome might relate to the Smad complex activation and duration of TGF-beta1 exposure. Further dissect the role of WWOX/WOX1 in TGF-beta induced TIAF1 self-aggregation and Smad4 overexpression in senile plaques formation might shed light for the development of therapeutic strategy in AD.

3.9. Conclusion and the future for WWOX/WOX1 in AD research

The interaction between WWOX/WOX1 and its binding partners determine the functioning roles of WWOX/WOX1 in neurodegeneration. Physical interaction of WWOX/WOX1 with Tau at the ADH/SDR domain and JNK at first WW domain regulate Tau phosphorylation *in vitro* and *in vivo* (22, 32). Knocked-down of WWOX/WOX1 expression by siRNA in SK-N-SH cells enhances Tau phosphorylation along with enhanced phosphorylation of GSK3-beta and ERK, and NFT formation (32). Ectopic expression of ADH/SDR domain of WWOX/WOX1 suppresses E2-induced Tau phosphorylation and increases TNF cytotoxicity *in vitro* (32*)*. The findings suggest that WWOX/WOX1 prevents phosphorylation of Tau by interacting with JNK1, GSK-3, and other enzymes *in vivo*. Hyperactive GSK3-beta in AD leads to hyperphosphorylation of Tau. GSK3-beta also regulates APP metabolism, and is conducive to amyloidogenic cleavage (7). WWOX/WOX1 through sequestration of Dvl protein inhibits Wnt/beta-catenin pathway (26). Wnt signaling contributes to amyloid beta peptide-mediated neuropathology in AD by inactivate GSK, which prevents Tau phosphorylation in the GSK-dependent epitopes (96, 100). GSK3-beta initiates proteosomal degradation of beta-catenin by phosphorylation of beta-catenin on key residues. Also, GSK3-beta has a nuclear function in downregulating the activity of beta-catenin (134). It is apparent that WWOX/WOX1 might directly or indirectly partner with aforementioned signal transduction pathways to participate in the overloaded amyloid beta-induced Tau hyperphosphorylation and neural dysfunction or death, which is critical in the pathogenesis of AD. With these considerations in regard, downregulating or inhibiting WWOX/WOX1 expression might be used as a therapeutic intervention strategy in AD. However, because WWOX/WOX1 is also a tumor suppressor, whether this approach will induce tumorigensis requires further consideration.

Obesity, high cholesterol, and diabetes increase the risk of dementia. Those are problems on the rise in many developing countries and are areas of focus in AD research. WOX1/WWOX is associated with low plasma HDL C levels and the *WWOX* gene is associated with HDL cholesterol and triglyceride levels which are crucial for the development of metabolic syndrome, and increases the risk for AD (37-39). Multiple metabolic defects occur in WWOX/WOX1-knockout mice suggesting WWOX/WOX1 may be a key regulator in different metabolic processes (40-42, 135). Research of WW domain-containing proteins on metabolic syndrome may help to further understand the role of WWOX/WOX1 in AD.

In conclusion, WWOX/WOX1, through interactions with its protein partners, plays important roles as multitasked protein in regulating apoptosis, cell growth and proliferation, DNA damage/ repair, cell trafficking, metabolic reactions, and central nervous system pathology and degeneration, any one of which might play an important role in the pathogenesis of AD (25, 35, 36, 135). This review only touches the tip of iceberg for the potential roles of WWOX/WOX1 in AD. We hope this review will bring up more researches to explore the role of WWOX/WOX1 in AD and other neurodegenerative diseases.

4. ACKNOWLEDGEMENTS

5. REFERENCES

1. G. A. Jicha and S. A. Carr: Conceptual evolution in Alzheimer's disease: Implications for understanding the clinical phenotype of progressive. *J Alzheimers Dis*, 19(1), 253–272 (2010)

2. C. Reitz, C. Brayne, and R. Mayeux: Epidemiology of Alzheimer disease. *Nature Reviews Neurology* 7 (3), 137-152 (2011)

3. H. Braak and E. Braak: Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* (Berl), 82 (4), 239–259 (1991)

4. K.P. Riley, D. A. Snowdon, and W.R. Markesbery: Alzheimer's neurofibrillary pathology and the spectrum of cognitive function: findings from the Nun Study. *Ann Neurol*, 51(5), 567–577 (2002)

5. P. Penzes and J. E.Vanleeuwen: Impaired regulation of synaptic actin cytoskeleton in Alzheimer's disease. *Brain Res Rev*, 67(1-2), 184-192 (2011)

6. L. M. Ittner and J. Götz: Amyloid-beta and tau--a toxic pas de deux in Alzheimer's disease. *Nat Rev Neurosci*, 12(2), 65-72 (2010) Review.

7. VJR. de Paula1, F M. Guimarães, B. S. Diniz, and O. V.Forlenza: Neurobiological pathways to Alzheimer's disease Amyloid-beta, Tau protein or both? *Dementia & Neuropsychologia*, 3(3), 188-194 (2009) Views & review

8. H.C. Huang and Z. F. Jiang: Accumulated amyloid-beta peptide and hyperphosphorylated tau protein: relationship and links in Alzheimer's disease. *Journal of Alzheimer's Disease*, 16, 15–27 (2009)

9. D. Muyllaert, A. Kremer, T. Jaworski, P. Borghgraef, H .Devijver, S. Croes, I. Dewachter, F. Van Leuven: Glycogen synthase kinase-3beta, or a link between amyloid and tau pathology? *Genes Brain Behav*, 7 (Suppl 1), 57-66 (2008)

10. Z. F. Wang, H. L. Li, X. C. Li, Q. Zhang, Q. Tian, Q. Wang, H. Xu, J. Z. Wang: Effects of endogenous beta-amyloid overproduction on tau phosphorylation in cell culture. *J Neurochem*, 98, 1167-1175 (2006)

11. P. Hernandez, G. Lee, M. Sjoberg, and R. B. Maccioni: Tau phosphorylation by cdk5 and Fyn in response to amyloid peptide Abeta 25−35: involvement of lipid rafts. *Journal of Alzheimer's Disease*, 16 (1), 149–156(2009)

12. G. Amadoro, V. Corsetti, M.T. Ciotti, F. Florenzano, S. Capsoni, G. Amatob, and P. C alissano: Endogenous Abeta causes cell death via early tau hyperphosphorylation. *Neurobiol Aging*, 1-22 (2009)

13. Y. Zhang , Q. Tian, Q. Zhang, X. Zhou, S. Liu, and J.Z.Wang: Hyperphosphorylation of microtubule-associated tau protein plays dual role in neurodegeneration and neuroprotection. *Pathophysiology*, 16,311–316 (2009)

14. L. Buée, L. Troquier, S. Burnouf, K. Belarbi, A.Van der Jeugd, T. Ahmed, F. Fernandez- Gomez, R. Caillierez, M. E. Grosjean, S. Begard, B. Barbot, D. Demeyer, H. Obriot, I. Brion, V. Buée-Scherrer, C. A. Maurage, D. Balschun, R. D'hooge, M. Hamdane, D. Blum, and N. Sergeant: From tau phosphorylation to tau aggregation: what about neuronal death? *Biochem Soc Trans*, 38(4), 967-72 (2010)

15. A. K. Bednarek, K. J. Laflin, R. L. Daniel, Q. Liao, K. A. Hawkins, and C. M. Aldaz: WWOX, a novel WW domain-containing protein mapping to human chromosome 16q23.3-24.1, a region frequently affected in breast cancer. *Cancer Res*, 60, 2140-2145 (2000).

16.K. Reid, M. Finnis, L. Hobson, M. Mangelsdorf, S. Dayan, J. K. Nancarros, E. Woollatt, G. Kremmidiotis, A. Gardner, D.Venter, E.Baker, and R.I.Richards: Common chromosomal fragile site FRAD16D sequence: identification for the FOR gene spanning FRAD16D and homozygous deletions and translcoation breakpoints in cancer cells. *Hum Mol Genet*. 9(11), 1651-1663 (2000)

17.N. S. Chang, J. Doherty, A. Ensign, J. Lewis, J. Heath, L. Schultz, S. T. Chen, and U. Oppermann: Molecular mechanisms underlying WOX1 activation during apoptotic

and stress responses. *Biochem Pharmacol*, 66(8), 1347-54 (2003) Review.

18. K. Huebner and C. M. Croce: FRA3B and other common fragile sites: the weakest links. *Nat Rev Cancer*, 1(3), 214-221 (2001)

19. N. S. Chang, J. Doherty, A. Ensign, L. Schultz, L .J. Hsu, and Q Hong: WOX1 is essential for tumor necrosis factor-, UV light-, staurosporine-, and p53-mediated cell death, and its tyrosine 33-phosphorylated form binds and stabilizes serine 46-phosphorylated p53. *J Biol Chem*, 280(52), 43100-8 (2005)

20. S. Strano, E. Munarriz, M. Rossi, L. Castagnoli, Y. Shaul, A. Sacchi, M. Oren, M. Sudol, G. Cesareni, and G.Blandino: Physical interaction with Yes-associated protein enhances p73 transcriptional activity. *J Biol Chem*, 276(18), 15164–15173 (2001)

21. A. Komuro, M. Nagai, N. E. Navin, and M. Sudol: WW domain-containing protein YAP associates with ErbB-4 and acts as a co-transcriptional activator for the carboxyl-terminal fragment of ErbB-4 that translocates to the nucleus. *J Biol Chem*, 278(35), 33334–33341 (2003)

22. N. S. Chang, J. Doherty, and A. Ensign: JNK1 physically interacts with WW domain- containing oxidoreductase (WOX1) and inhibits WOX1-mediated apoptosis. *J Biol Chem*, 278(11), 9195-202 (2003)

23. Q. Hong, L. J. Hsu, L. Schultz, N. Pratt, J. Mattison, and N. S. Chang: Zfra affects TNF-mediated cell death by interacting with death domain protein TRADD and negatively regulates the activation of NF-kappaB, JNK1, p53 and WOX1 during stress response. *BMC Mol Biol,* 8,50 (2007)

24. K .C. Kurek, S. Del Mare, Z. Salah, S. Abdeen, H. Sadiq, S. H. Lee, E. Gaudio, N. Zanesi, K. B. Jones, B. DeYoung, G. Amir, M. Gebhardt, M. Warman, G. S. Stein, J. L. Stein, J. B. Lian, and R. I. Aqeilan: Frequent attenuation of the WWOX tumor suppressor in osteosarcoma is associated with increased tumorigenicity and aberrant RUNX2 expression. *Cancer Res*, 70(13), 5577-86 (2010)

25. R. I. Aqeilan, M. Q. Hassan, A. de Bruin, J. P. Hagan, S. Volinia, T. Palumbo, S. Hussain, S. H. Lee, T. Gaur, G. S. Stein, J. B. Lian, and C. M. Croce: The WWOX tumor suppressor is essential for postnatal survival and normal bone metabolism. *J Biol Chem,* 283(31), 21629-39 (2008)

26. N. Bouteille, K. Driouch, P. E. Hage, S. Sin, E. Formstecher, J. Camonis, R. Lidereau, and F. Lallemand: Inhibition of the Wnt/beta-catenin pathway by the WWOX tumor suppressor protein. *Oncogene*, 28(28), 2569-80 (2009)

27. C. Jin, L. Ge, X. Ding, Y. Chen, H. Zhu, T. Ward, F. Wu, X. Cao, Q. Wang, and X. Yao: PKA-mediated

protein phosphorylation regulates ezrin-WWOX interaction. *Biochem Biophys Res Commun*, 341(3), 784-91 (2006)

28. N. S. Chang, N. Pratt, J. Heath, L Schultz, D. Sleve, G. B. Carey, and N. Zevotek: Hyaluronidase induction of a WW domain-containing oxidoreductase that enhances tumor necrosis factor cytotoxicity. *J Biol Chem*, 276(5), 3361-3370 (2001)

29. R.J. Davis: Signal transduction by the JNK group of MAP kinases. *Cell*, 103(2), 239-252 (2000)

30. A. Roulston, C. Reinhard, P. Amiri, and L. T. Williams: Early activation of c-Jun N-terminal kinase and p38 kinase regulate cell survival in response to tumor necrosis factor alpha. *J Biol Chem*, 273(17), 10232-10239 (1998)

31. G. V. De Ferrari and N. C. Inestrosa: Wnt signaling function in Alzheimer's disease. *Brain Research Reviews*, 33(1), 1-12(2000)

32. C. I. Sze, S. Meng, S. Pugazhenthi, P. Jambal, L. J. Hsu, J. Heath, L. Schultz, and N. S. Chang: Down-regulation of WW Domain-containing oxidoreductase induces tau phosphorylation *in vitro*. *J Biol Chem*, 279(29), 30498–30506 (2004)

33. N. S. Chang: A potential role of p53 and WOX1 in mitochondrial apoptosis. *Int J Mol Med*, 9(1), 19 24 (2002) Review.

34. N. S. Chang, L. J. Hsu, Y. S. Lin, F. J. Lai, and H. M. Sheu: WW domain-containing oxidoreductase: a candidate tumor suppressor. *Trends Mol Med*, 13(1), 12 22 (2007)

35. J. Y. Chang, R.Y. He, H. P. Lin, L. J. Hsu, F. J. Lai, Q. Hong, S. J. Chen, and N. S. Chang: Signaling from membrane receptors to tumor suppressor WW domain-containing oxidoreductase. *Exp Biol Med*, (Maywood) 235(7), 796 804 (2010) Review.

36. R. I. Aqeilan and C. M. Croce: WWOX in biological control and tumorigenesis. *J Cell Physiol*, 212(2), 307 310 (2007)

37. J. C. Lee, D. Weissglas Volkov, M. Kyttälä, Z. Dastani, R. M. Cantor, E. M. Sobel, *et al*: WW domain containing oxidoreductase is associated with low plasma HDL C levels. *Am J Hum Genet*, 83(2), 180 192 (2008)

38. M. E. Sáez, A. González-Pérez, M. T. Martínez-Larrad, J. Gayán, L .M. Real, M. Serrano-Ríos, and A. Ruiz: WWOX gene is associated with HDL cholesterol and triglyceride levels.*BMC Med Genet*, 11,148 (2010)

39. Z. Dastani, P. Pajukanta, M. Marcil, N. Rudzicz, I. Ruel, S .D. Bailey, J. C. Lee, M. Lemire, J. Faith, J. Platko, J. Rioux, T. J. Hudson, D. Gaudet, J. C. Engert, and J. Genest: Fine mapping and association studies of a high-density lipoprotein cholesterol linkage region on chromosome 16 in French-Canadian subjects. *Euro J Hum Genet*, 18, 342–347 (2010)

40. D. Iliopoulos, K. N. Malizos, P. Oikonomou, and A. Tsezou: Integrative microRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. *PLoS One*, 3 (11): e3740 (2008)

41. J. H. Ludes Meyers, H. Kil, J. Parker Thornburg, D. F. Kusewitt, M. T. Bedford, and C. M. Aldaz: Generation and characterization of mice carrying a conditional allele of the Wwox tumor suppressor gene. *PLoS One*, 4 (11): e7775 (2009)

42. L. V. Oõkeefe, A. Colella, S. Dayan, Q. A. Chen Choo, R. Jacob, *et al.* Drosophila orthologue of WWOX, the chromosomal fragile site FRA16D tumour suppressor gene, functions in aerobic metabolism and regulates reactive oxygen species. *Hum Mol Genet.* (2010) in press

43. S. T. Chen, J. I. Chuang, J. P. Wang, M .S. Tsai, H. Li, and N. S. Chang: Expression of WW domain containing oxidoreductase WOX1 in the developing murine nervous system. *Neuroscience*, 124(4), 831 839 (2004)

44. S. T. Chen, J. I. Chuang, C. L. Cheng, L. J. Hsu, and N. S. Chang: Light induced retinal damage involves tyrosine 33 phosphorylation, mitochondrial and nuclear translocation of WW domain containing oxidoreductase *in vivo*. *Neuroscience*, 130(2), 397 407 (2005)

45. C. P. Lo, L. J. Hsu, M. Y.Li, S. Y. Hsu, J. I. Chuang, M. S.Tsai, S. R. Lin, N. S. Chang, and S. T. Chen: MPP+ induced neuronal death in rats involves tyrosine 33 phosphorylation of WW domain containing oxidoreductase WOX1. *Eur J Neurosci*, 27(7), 1634 1646 (2008)

46. M. Y. Li, F. J. Lai, L .J. Hsu, C. P. Lo, C. L. Cheng, M .S. Tsai, C. I Sze, S. Pugazhenthi, N. S. Chang, and S. T. Chen: Dramatic co activation of WOX1 with CREB and NF kB in delayed loss of small dorsal root ganglion neurons upon sciatic nerve transection in rats. *PLoS One*, 4: e7820 (2009)

47. H. Suzuki, K. Katayama, M. Takenaka, K. Amakasu, K. Saito, and K. Suzuki: A spontaneous mutation of the Wwox gene and audiogenic seizures in rats with lethal dwarfism and epilepsy. *Genes Brain Behav*, 8 (7), 650 660 (2009)

48. C. Chen and L. E. Matesic: The Nedd4-like family of E3 ubiquitin ligases and cancer. *Cancer Metast Rev*, 26 (3-4), 587–604 (2007)

49. L. J. Hsu, L. Schultz, Q. Hong, K. Van Moer, J. Heath, M. Y. Li, F. J. Lai, S. R. Lin, M .H. Lee, C. P. Lo, Y. S. Lin, S. T. Chen, and N .S. Chang: Transforming growth factor beta1 signaling via interaction with cell surface Hyal-2 and recruitment of WWOX/WOX1. *J Biol Chem*, 284,16049-16059 (2009)

50. P. Bork and M. Sudol: The WW domain: a signalling site in dystrophin? *Trends Biochem Sci,* 19, 531–533 (1994).

51. O. Staub and D. Rotin: WW Domains. *Structure* (Curr. Biol.), 4, 495–499(1996)

52. M. J. Macias, M. Hyvo¨nen, E. Baraldi, J. Schultz, M.Sudol, M. Saraste and H. Oschkinat: Structure of the WW domain of a kinase-associated protein complexed with a proline-rich peptide. *Nature*, 382, 646–649 (1996)

53. H. I. Chen, A. Einbond, S. J. Kwak, H. Linn, E. Koepf, S. Peterson, J. W. Kelly and M. Sudol: Characterization of the WW domain of human Yes-associated protein and its polyproline- containing ligands. *J Biol Chem*, 272, 17070–17077 (1997)

54. E. K. Koepf, H. M. Petrassi1, M. Sudol, and J. W. Kelly: WW: An isolated three-stranded antiparallel beta-sheet domain that unfolds and refolds reversibly; evidence for a structured hydrophobic cluster in urea and GdnHCl and a disordered thermal unfolded state. *Protein Science*, 8(4), 841-853 (1999)

55. R. J. Ingham, K. Colwill, C. Howard, S. Dettwiler, C. S. H. Lim, J. Yu, K. Hersi, J. Raaijmakers, G. Gish, G. Mbamalu, L. Taylor, B. Yeung, G. Vassilovski, M. Amin, F. Chen, L. Matskova, G. Winberg, I. Ernberg, R. Linding, P. O'Donnell, A. Starostine, W. Keller, P. Metalnikov, C. Stark, and T. Pawson: WW Domains provide a platform for the assembly of multiprotein networks. *Mol Cell Biol*, 25(16), 7092–7106 (2005)

56. M. Sudol, C. C. Recinos, J. Abraczinskas, J. Humbert, and A. Farooq: WW or WoW: the WW domains in a union of bliss. *IUBMB Life*, 7,773–8 (2005)

57. K. Wang, C. Degerny, M. Xu, and X. J.Yang: YAP, TAZ, and Yorkie: a conserved family of signal-responsive transcriptional coregulators in animal development and human disease. *Biochem Cell Biol*, 87(1), 77–91(2009)

58. N. S. Chang, L. Schultz, L. J. Hsu, J. Lewis, M. Su, and C. I. Sze: 17beta-Estradiol upregulates and activates WOX1/WWOXv1 and WOX2/WWOXv2 *in vitro*: potential role in cancerous progression of breast and prostate to a premetastatic state *in vivo*. *Oncogene*, 24(4), 714–23 (2005)

59. Q. Hong, C. I. Sze, S. R. Lin, M .H. Lee, R. Y. He, L. Schultz, J. Y. Chang, S. J. Chen, R. J. Boackle , L .J. Hsu, N .S. Chang: Complement C1q activates tumor suppressor WWOX to induce apoptosis in prostate cancer cells. *PLoS One*, 4(6):e5755 (2009)

60. W. Chan, R. Tian, Y. F. Lee, S. T. Sit, L. Lim, and E. Manser: Down-regulation of active ACK1 is mediated by association with the E3 ubiquitin ligase Nedd4-2. *J Biol Chem*, 284(12), 8185–94(2009)

61. J. Y. Chang, R. Y. He, H. P. Lin, L. J. Hsu, F. J. Lai, Q. Hong, S. J. Chen, and N. S. Chang: Signaling from membrane receptors to tumor suppressor WW domain-containing oxidoreductase. *Exp Biol Med*, 235(7), 796-804 (2010)

62. A.N. Hegde and S.C. Upadhya: The ubiquitin–proteasome pathway in health and disease of the nervous system. *TRENDS in Neurosciences*, 30(11), 587-595 (2007)

63. Y. Kato, K. Nagata, M. Takahashi, L. Lian, J. J. Herrero, M. Sudol, and M. Tanokura: Common mechanism of ligand recognition by group II/III WW domains: redefining their functional classification. *J Biol Chem*, 279(30), 31833–41(2004)

64. M. I. Behrens, C. Lendon, and C. M. Roe: A common biological mechanism in cancer and Alzheimer's disease? *Curr Alzheimer Res*, 6 (3), 196–204 (2009)

65. G. Finn and K. P. Lu: Phosphorylation-specific prolyl isomerase Pin1 as a new diagnostic and therapeutic target for cancer. *Curr Cancer Drug Targets*, 8(3), 223–9 (2008)

66. S. Esnault, Z. J. Shen, and J .S. Malter: Pinning down signaling in the immune system: the role of the peptidyl-prolyl isomerase Pin1 in immune cell function. *Crit Rev Immunol*, 28(1), 45–60 (2008)

67. H. L. Li, H. H. Wang, S. J .Liu, Y. Q. Deng, Y.J. Zhang, Q. Tian, X.C. Wang, X.Q. Chen, Y. Yang, J. Y. Zhang, J. Y. Wang, H. Xu, F.F. Liao, and J. Z. Wang: Phosphorylation of tau antagonizes apoptosis by stabilizing beta-catenin, a mechanism involved in Alzheimer's neurodegeneration. *Proc Natl Acad Sci USA*, 104(9), 3591–3596 (2007)

68. G. Drewes, B. Trinczek, S. Illenberger, J. Biernat, G. Schmitt-Ulms, H. E. Meyer,E. M. Mandelkow, and E. Mandelkow: Microtubule-associated protein/microtubule affinity-regulating kinase (p110mark). A novel protein kinase that regulates tau-microtubule interactions and dynamic instability by phosphorylation at the Alzheimer-specific site serine 262. *J Biol Chem*, 270(13), 7679–7688 (1995)

69. F. Liu, K. Iqbal, I. Grundke-Iqbal, G. W. Hart, and C. X. Gong: O-GlcNAcylation regulates phosphorylation of tau: amechanism involved in Alzheimer's disease. *Proc Natl Acad Sci U.S.A*, 101, 10804–10809 (2004a)

70. S. J. Liu, J .Y. Zhang, H. L. Li, Z. Y. Fang, Q. Wang, H. M. Deng, C. X. Gong, I. Grundke- Iqbal, K. Iqbal, and J. Z. Wang: Tau becomes amore favorable substrate for GSK-3 when it is prephosphorylated by PKA in rat brain. *J Biol Chem*, 279(48), 50078–50088 (2004b)

71. C .H. Reynolds, J. C. Betts, W. P. Blackstock, A. R. Nebreda, and B. H. Anderton:
Phosphorylation sites on tau identified by nanoelectrospray mass spectrometry:differences *in vitro* between the mitogen-activated protein kinases ERK2, c-Jun N-terminal kinase and P38, and glycogen synthase kinase-3beta. *J Neurochem*, 74(4), 1587–1595(2000)

72. T. J. Singh, J. Z. Wang, M. Novak, E. Kontzekova, I. Grundke-Iqbal, and K. Iqbal: Calcium/calmodulin-dependent protein kinase II phosphorylates tau at Ser-262

but only partially inhibits its binding to microtubules. *FEBS Lett*, 387(2-3), 145–148 (1996)

73. H. Yamamoto, Y. Hiragami, M. Murayama, K. Ishizuka, M. Kawahara, and A. Takashima: Phosphorylation of tau at serine 416 by Ca2+/calmodulin-dependent protein kinase II in neuronal soma in brain. *J Neurochem*, 94(5), 1438–1447(2005)

74. T. Horiguchi, K. Uryu, B. I. Giasson, H. Ischiropoulos, R. LightFoot, C. Bellmann, C. Richter-Landsberg, V. M. Lee, and J. Q. Trojanowski: Nitration of tau protein is linked to neurodegeneration in tauopathies. *Am J Pathol*, 163(3), 1021- 1031(2003)

75. C. Richter-Landsberg and O. Goldbaum: Stress proteins in neural cells: functional roles in health and disease. *Cell Mol Life Sci*, 60(2), 337-49 (2003) Review.

76. J. N. Keller, K .B. Hanni, and W.R. Markesbery: Impaired proteasome function in Alzheimer'sdisease. *J Neurochem*, 75(1), 436–439 (2000)

77. Y. J. Zhang, Y. F. Xu, X. H. Liu, D. Li, J. Yin, Y. H. Liu, X.Q. Chen, and J.Z. Wang: Carboxyl terminus of heat-shock cognate 70-interacting protein degrades tau regardless it phosphorylation status without affecting the spatial memory of the rats. *J Neural Transmission*, 115(3), 483-491(2008)

78. J. J. Lucas, F. Herna´ ndez, P. Go´ mez-Ramos, M. A. Mora´ n, R. Hen, J. Avila: Decreased nuclear beta-catenin, tau hyperphosphorylation and neurodegeneration in GSK-3beta conditional transgenic mice. *EMBO J,* 20, 27–39 (2001)

79. L. Q. Zhu, S. H. Wang, D. Liu, Y. Y. Yin, Q. Tian, X. C. Wang, Q. Wang, J .G. Chen, and J .Z. Wang: Activation of glycogen synthase kinase-3 inhibits long-term potentiation with synapse-associated impairments. *J Neurosci*, 27,12211–12220 (2007)

80. Z. F. Wang, H. L. Li, X. C. Li, Q. Zhang, Q. Tian, Q. Wang, H. Xu, and J .Z. Wang: Effects of endogenous beta-amyloid overproduction on tau phosphorylation in cell culture. *J Neurochem*, 98(4), 1167–1175 (2006)

81. Y .J. Zhang, Y. F. Xu, Y. H. Liu, J. Yin, H. L. Li, Q. Wang, and J .Z. Wang: Peroxynitrite induces Alzheimer-like tau modifications and accumulation in rat brain and its underlying mechanisms. *FASEB J*, 20(9), 1431–1442 (2006)

82. J. Z. Wang and F. Liu: Microtubule-associated protein tau in development, degeneration and protection of neurons. *Progress in Neurobiology*, 85(2), 148–175(2008)

83. J. Avila, I. Santa-Marı´a, M. Pe´ rez, F. Herna´ ndez, and F. Moreno: Tau phosphorylation, aggregation, and cell toxicity. *J Biomed Biotechnol*, 2006,74539 (2006)

84. I. Khlistunova, J. Biernat, Y. Wang, M. Pickhardt, M. von Bergen, Z. Gazova, E. Mandelkow, and E. M. Mandelkow: Inducible expression of tau repeat domain in cell models of tauopathy: aggregation is toxic to cells but

can be reversed by inhibitor drugs. *J Biol Chem*, 281(2), 1205–1214 (2006)

85. Y. P. Wang, J. Biernat, M. Pickhardt, E. Mandelkow, and E .M. Mandelkow: Stepwise proteolysis liberates tau fragments that nucleate the Alzheimer-like aggregation of full-length tau in a neuronal cell model. *Proc Natl Acad Sci USA*, 104(24), 10252–10257(2007)

86. K. Santacruz, J. Lewis, T. Spires, J. Paulson, L. Kotilinek, M. Ingelsson, A. Guimaraes, M. DeTure, E. Ramsden McGowan, C. Forster, M. Yue, J. Orne, C. Janus, A. Mariash, M. Kuskowski, B. Hyman, M. Hutton, and H. Ashe: Tau suppression in a neurodegenerative mouse model improves memory function. *Science*, 309(5733), 476–481(2005)

87. A. C. Alonso, B. Li, I. Grundke-Iqbal, and K. Iqbal: Polymerization of hyperphosphorylated tau into filaments eliminates its inhibitory activity. *Proc Natl Acad Sci USA*, 103(23), 8864– 8869 (2006)

88. C. Andorfer, C. M. Acker, Y. Kress, P. R. Hof, K. Duff, and P. Davies: Cell-cycle reentry and cell death in transgenic mice expressing nonmutant human tau isoforms. *J Neurosci*, 25(22), 5446– 5454 (2005)

89. T. L. Spires, J. D. Orne, K. SantaCruz, R. Pitstick, G. A. Carlson, K. H. Ashe, and B. T. Hyman: Region-specific dissociation of neuronal loss and neurofibrillary pathology in a mouse model of tauopathy. *Am J Pathol*, 168(5), 1598–1607(2006)

90. A. Gomez-Ramos, J. Diaz-Nido, M. A. Smith, G. Perry, J. Avila: Effect of the lipid peroxidation product acrolein on tau phosphorylation in neural cells. *J Neurosci Res*, 71(6), 863–870 (2003)

91. A. Sultan, F. Nesslany, M.Violet, S. Be´gard, A. Loyens, S, Talahari, Z. Mansuroglu, D. Marzin, N. Sergeant, S. Humez, M. Colin,E. Bonnefoy, L. Bue´e, and M. C. Galas: Nuclear Tau, a Key Player in Neuronal DNA Protection. *J Biol Chem*, 286 (6), 4566–4575(2011)

92. H. G. Lee, G. Perry, P. I. Moreira, M. R. Garrett, Q. Liu, X. Zhu, A. Takeda, A. Nunomura, and M .A. Smith: Tau phosphorylation in Alzheimer's disease: pathogen or protector? *Trends Mol Med*, 11,164–169.

93. R. J. Castellani, H. G. Lee, X. Zhu, A. Nunomura, G. Perry, M .A. Smith: Neuropathology of Alzheimer disease: pathognomonic but not pathogenic. *Acta Neuropathol*, 111(6), 503–509 (2006)

94. M. H. Maurer, J.O. Brömme, R. E. Feldmann, Jr., A. Järve, F. Sabouri, H. F. Bürgers, D.W. Schelshorn, C. Krüger, A. Schneider, and W. Kuschinsky: Glycogen synthase kinase 3beta (GSK3beta) regulates differentiation and proliferation in neural stem cells from the rat subventricular zone. *J Proteome Res*, *6* (3), 1198–1208 (2007)

95. F. Massoud and S. Gauthier: Update on the Pharmacological Treatment of Alzheimer's Disease. *Curr Neuropharmacol*, 8(1), 69–80 (2010)

96. R. A. Boonen, P. van Tijn, and D. Zivkovic: Wnt signaling in Alzheimer's disease: up or down, that is the question. *Ageing Res Rev*, 8(2), 71-82 (2009)

97. W. Noble, E. Planel, C. Zehr, V.Olm, J. Meyerson, F. Suleman, K. Gaynor, L. Wang, J. LaFrancois, B. Feinstein, M. Burns, P.Krishnamurthy, Y. Wen, R. Bhat, J. Lewis, D.Dickson, and K. Duff: Inhibition of glycogen synthase kinase-3 by lithium correlates with reduced tauopathy and degeneration *in vivo*. *Proc Natl Acad Sci USA*, 102, 6990-6995 (2005)

98. Y. Wang, J. X. Zhang, X. X. Du, L. Zhao, Q. Tian, L .Q. Zhu, S. H. Wang, J. Z. Wang: Temporal correlation of the memory deficit with Alzheimer-like lesions induced by activation of glycogen synthase kinase-3. *J Neurochem*, 106(6), 2364-2374(2008)

99. O. V. Forlenza, J. M. Spink, R. Dayanandan, B. H. Anderton, O. F. Olesen, and S. Lovestone: Muscarinic agonists reduce tau phosphorylation in non-neuronal cells via GSK-3beta inhibition and in neurons. *J Neural Transm* 107(10), 1201-1212 (2000)

100. R. V. Bhat and S. L.Budd: GSK3beta signaling: casting a wide net in Alzheimer's disease. *Neurosignals*, 11(5), 251-261(2002)

101. M. Rapoport, H. N. Dawson, L. I. Binder, M. P. Vitek, and A. Ferreira: Tau is essential to beta-amyloid-induced neurotoxicity. *Proc Natl Acad Sci U S A*, 99(9), 6364-6369(2002)

102. R. W. Shin, K. Ogino, A. Shimabuku, T. Taki, H. Nakashima, T. Ishihara, and T. Kitamoto: Amyloid precursor protein cytoplasmic domain with phospho-Thr668 accumulates in Alzheimer's disease and its transgenic models: a role to mediate interaction of Abeta and tau. *Acta Neuropathol*, 113(6), 627-636(2007)

103. D. Muyllaert, A. Kremer, T. Jaworski, P. Borghgraef, H. Devijver, S. Croes, I. Dewachter, and F. Van Leuven: Glycogen synthase kinase-3beta, or a link between amyloid and tau pathology? *Genes Brain Behav*, 7 (Suppl 1), 57-66(2008)

104. T. Engel, J. J. Lucas, P. G´omez-Ramos, M .A. Moran, J. Avila, and F. Hern´andez: Cooexpression of FTDP-17 tau and GSK-3beta in transgenic mice induce tau polymerization and neurodegeneration. *Neurobiol Aging*, 27(9), 1258-1268 (2006)

105. K. Leroy, Z. Yilmaz, and J. P. Brion: Increased level of active GSK-3beta in Alzheimer's disease and accumulation in argyrophilic grains and in neurons at different stages of neurofibrillary degeneration. *Neuropathol Appl Neurobiol*, 33(1), 43-55 (2007)

106. Y. T. Lin, J. T. Cheng, L. C. Liang, C. Y. Ko, Lo YK, and P. J. Lu: The binding and phosphorylation of Thr231 is critical for Tau's hyperphosphorylation and functional regulation by glycogen synthase kinase 3beta. *J Neurochem*, 103(2), 802-813(2007)

107. S. Miranda, C. Opazo, L. F. Larrondo, F. J. Muˇnoz, F. Ruiz, F. Leighton, and N. C. Inestrosa: The role of oxidative stress in the toxicity induced by amyloid beta-peptide in Alzheimer's disease. *Prog Neurobiol*, 62(6), 633-648(2000)

108. D. A. Butterfield, M. Perluigi, and R. Sultana: Oxidative stress in Alzheimer's disease brain: new insights from redox proteomics. *Eur J Pharmacol*, 545(1), 39-50 (2006)

109. I .G. Onyango and S. M. Khan: Oxidative stress, mitochondrial dysfunction, and stress signaling in Alzheimer's disease. *Curr Alzheimer Res* 3(4), 339-349(2006)

110. H. Kadowaki, H. Nishitoh, F. Urano, C. Sadamitsu, A. Matsuzawa, K. Takeda, H. Masutani, J. Yodoi, Y. Urano, T. Nagano, and H. Ichijo: Amyloid beta induces neuronal cell death through ROS-mediated ASK1 activation. *Cell Death Differ*, 12(1), 19-24(2005)

111. C. Atzori, B. Ghetti, R. Piva, A. N. Srinivasan, P. Zolo, M. B. Delisle, S. S. Mirra, and A. Migheli: Activation of the JNK/p38 pathway occurs in diseases characterized by tau protein pathology and is related to tau phosphorylation but not to apoptosis. *J Neuropathol Exp Neurol*, 60(12), 1190-1197(2001)

112. M. J. Savage, Y. G. Lin, J .R. Ciallella, D. G. Flood, and R. W. Scott: Activation of c-Jun N-terminal kinase and p38 in an Alzheimer's disease model is associated with amyloid deposition. *J Neurosci*, 22(9), 3376-3385(2002)

113. H. Lassmann, C. Bancher, H. Breitschopf, J. Wegiel, M. Bobinski, K. Jellinger, and H. M. Wisniewski: Cell death in Alzheimer's disease evaluated by DNA fragmentation *in situ*. *Acta Neuropathol*, 89(1), 35-41(1995)

114. D. Y .Loo, A. Copani, C. J. Pike, E. R. Whittemore, A. J. Walencewicz, and C. W. Cotman: Apoptosis is induced by beta-mamyloid in cultured central nervous system neurons. *Proc Natl Acad Sci U S A*, 90, 7951-7955(1993)

115. K. Agerman, C. Baudet, B. Fundin, C. Willson, and P. Ernfors: Attenuation of a caspase-3 dependent cell death in NT4- and p75-deficient embryonic sensory neurons. *Mol Cell Neurosci*, 16(3), 258–268(2000)

116. K. T. Akama, C. Albanese, R. G.. Pestell, and L. J. Van Eldik: Amyloid b-peptide stimulates nitric oxide production in astrocytes through an NFkappaB-dependent mechanism. *Proc Natl Acad Sci U S A*, 95(10), 5795–5800(1998).

117. O. Ghribi, M. M. Herman, D. A. DeWitt, M. S. Forbes, and J. Savory: Ab (1–42) and aluminum induce stress in the endoplasmic reticulum in rabbit hippocampus, involving nuclear translocation of gadd 153 and NF-κB. *Brain Res Mol Brain Res*, 96(1-2), 30–38(2001)

118. Y. Shen, P. He, Z. Zhong, C. McAllister, and K. Lindholm: Distinct destructive signaling pathways of neuronal death in Alzheimer's disease. *TRENDS in Molecular Medicine*, 12(12), 574-579(2006)

119. T. T. Rohn, R. A. Rissman, E. Head, and C.W. Cotman: Caspase activation in the Alzheimer's disease brain: tortuous and torturous. *Drug News Perspect*, 15(9), 549–557(2002).

120. C. Lanni, L. Nardinocchi, R. Puca, S. Stanga, D. Uberti, M. Memo, S. Govoni, G. D'Orazi, and M. Racchi: Homeodomain interacting protein kinase 2: A target for Alzheimer's beta amyloid leading to misfolded p53 and inappropriate cell survival. *PLoS ONE*, 5(4): e10171(2010)

121. S. Stanga, C.Lanni, S. Govoni, D. Uberti, G. D'Orazi, and M. Racchi: Unfolded p53 in the pathogenesis of Alzheimer's disease: is HIPK2 the link? *Aging*, 2 (9), 545-554(2010)

122. C. Lanni, M. Racchi, G. Mazzini, A. Ranzenigo, R. Polotti, E. Sinforiani, L. Olivari, M. Barcikowska, M. Styczynska, J. Kuznicki, A. Szybinska, S. Govoni, M. Memo, and D. Uberti: Conformationally altered p53: a novel Alzheimer's disease marker? *Molecular Psychiatry*, 13, 641–647(2008)

123. H. Jiang, D. Burdick, C. G. Glabe, C. W. Cotman, and A. J. Tenner: Beta-Amyloid activates complement by binding to a specific region of the collagen-like domain of the C1q A chain. *J Immunol* 152(10), 5050–5059(1994)

124. M. I. Fonseca, S. H. Chu, A. M. Berci, M.E. Benoit, D. G. Peters, Y. Kimura, and A. J. Tenner: Contribution of complement activation pathways to neuropathology differs among mouse models of Alzheimer's disease. *J Neuroinflammation*, 8(4), 1-12 (2011)

125. A. J. Tenner: Complement in Alzheimer's disease: opportunities for modulating protective and pathogenic events. *Neurobiol Aging*, 22(6), 849–861(2001)

126. Y. Shen, L. Lue, L. Yang, A. Roher, Y. Kuo, R. Strohmeyer, W. J. Goux , V. Lee, G. V. Johnson , S. D. Webster, N. R. Cooper, B. Bradt, and J .Rogers: Complement activation by neurofibrillary tangles in Alzheimer's disease. *Neurosci Lett*, 305(3), 165-168(2001)

127. A. Afagh, B. J. Cummings, D. H. Cribbs, C. W. Cotman, and A. J. Tenner: Localization and cell association of C1q in Alzheimer's disease brain. *Exp Neurol*, 138(1), 22-32(1996)

128. S. E. Stoltzner, T. J. Grenfell, C. Mori, K. E. Wisniewski, T. M. Wisniewski,D. J. Selkoe, and C. A.

Lemere: Temporal accrual of complement proteins in amyloid plaques in Down's syndrome with Alzheimer's disease. *Am J Pathol*, 156(2), 489– 499(2000)

129. M. H. Lee, S .R. Lin, J. Y. Chang, L. Schultz, J. Heath, L. J. Hsu, Y. M. Kuo, Q. Hong, M. F. Chiang, C. X. Gong, C. I. Sze, and N-S Chang: TGF-beta induces TIAF1 self-aggregation via type II receptor-independent signaling that leads to generation of amyloid beta plaques in Alzheimer's disease. *Cell Death and Disease*, 1, e110 (2010)

130. T. Burton, B. Liang, A. Dibrov, and F. Amara: Transforming growth factor-beta-induced transcription of the Alzheimer beta-amyloid precursor protein gene involves interaction between the CTCF-complex and Smads. *Biochem Biophys Res Commun*, 295(3), 713– 723(2002)

131. F. Docagne, C. Gabriel, N. Lebeurrier, S. Lesne, Y. Hommet, L. Plawinski, E. T. Mackenzie, and D. Vivien: Sp1 and Smad transcription factors co-operate to mediate TGF-beta-dependent activation of amyloid-beta precursor protein gene transcription. *Biochem J*, 383(pt-2), 393– 399 (2004)

132. U. Ueberham, E. Ueberham, M. K. Bruckner, G. Seeger, U. Gartner, H. Gruschka, R. Gebhardt, T. Arendt: Inducible neuronal expression of transgenic TGF-beta1 *in vivo*: dissection of short-term and long-term effects. *Eur J Neurosci*, 22(1), 50–64(2005)

133. A. G. Henriques, S. I. Vieira, E. F. da Cruz e Silva, and O. A. B. da Cruz e Silva: beta hinders nuclear targeting of AICD and Fe65 in primary neuronal cultures. *J Mol Neurosci*, 39, 248– 255(2009)

134. Caspi M, Zilberberg A, Eldar-Finkelman H, Rosin-Arbesfeld R. Nuclear GSK-3beta inhibits the canonical Wnt signaling pathway in a beta-catenin phosphorylation - independent manner. *Oncogene*, 27(25), 3546-55(2008)

135. S. Del Mare, Z. Salah, and R. I. Aqeilan: WWOX: Its Genomics, Partners, and Functions. *J Cell Biochem*, 108, 737–745(2009)

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