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Review article

**Post-translational modifications of the p53 protein and the impact in Alzheimer's disease: a review of the literature**

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# Abstract

Our understanding of Alzheimer's disease (AD) pathogenesis has developed with several hypotheses over the last 40 years, including the Amyloid and Tau hypotheses, respectively. More recently, the p53 protein, well-known as 'the guardian of the genome,' has gained attention for its role in the early evolution of AD. This is due to p53's central role in the control of oxidative stress and potential involvement in both Amyloid and Tau pathways. p53 is

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commonly regulated by post-translational modifications (PTMs), which affect its conformation, increasing its capacity to adopt multiple structural and functional states, including those that can influence several processes in AD. The following review will explore the impact of p53 post-translational modifications (PTMs) on its function and consequential involvement in AD pathogenesis.

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# Introduction

## Pathology of Alzheimer's Disease

Alzheimer's disease (AD) is a devastating condition of the aging population that leads to progressive degeneration of brain function, whose characteristic anatomopathological hallmarks, firstly described by Alois Alzheimer, remain the senile plaques and neurofibrillary tangles consisting of beta-amyloid peptides and hyperphosphorylated Tau protein, respectively (Querfurth and LaFerla 2010). The formation of brain plaques begins up to 20 years before any signs of clinical symptoms. To date, our understanding of AD pathology is still incomplete, with several different models proposed (Jack and Holtzman 2013; Fagan et al., 2014).

The amyloid hypothesis of AD pathology suggests that the increased presence of amyloid-beta (Aβ) is the triggering factor, which then links directly to Tau protein hyper-phosphorylation, synapse loss, and cell death (Gao et al., 2018).

However, the classical amyloid hypothesis has been questioned (Ricciarelli and Fedele 2017), and additional hypotheses have been proposed to explain the pathophysiology of AD.

GSK-3β is a critical enzyme in regulating the cell cycle and plays a pivotal role in neurons in the regulation of Tau. Thus, increased GSK-3β activity is an early event in AD pathology, triggering a cascade of events culminating in increased production of Aβ and Tau hyper-phosphorylation (Braak and Del Tredici 2004). Moreover, the ε4 isoform of Apolipoprotein E (Apo E4), which inhibits proteolytic degradation of Aβ, has also been shown to be a

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significant genetic risk factor for the sporadic form of AD Mulder et al., 2014(Mulder et al., 2014). In addition, glutamatergic and cholinergic dysfunction (Howes and Houghton 2012), oxidative stress (Cheignon et al., 2018), prion proteins (Westergard, Christensen, and Harris 2007), α-synuclein (Twohig and Nielsen 2019), TDP-42 (Tomé et al., 2020) and inflammation (Latta, Brothers, and Wilcock 2015) are all implicated in the pathology of AD.

Mitochondrial dysfunction, accumulation of transition metals along with Aβ and Tau protein have all been implicated in the loss of redox balance, and oxidative stress is a typical characteristic of AD brains along with the presence of plaques and NFTs (Praticò 2008). In addition, damage by free radicals and alterations in the expression of critical antioxidant enzymes superoxide dismutase (SOD) and catalase have also been noted in tissue from AD patients (Marcus et al., 1998; Omar et al., 1999; Padurariu et al., 2010).

In the AD brain, fatty acid oxidation has been linked to Tau pathology, and p38 MAPK has been linked as a potential candidate gene within the relevant MAP Kinase pathway (Zhu et al., 2000). In addition, studies have implicated oxidative stress in Aβ induced neurotoxicity (Mattson 2007), and cellular (Behl et al., 1994) and rodent (Nishida et al., 2006; Li et al., 2004) models showed increased oxidative stress is linked to Aβ deposits.

The metal theory of AD (Bush 2013) proposes that homeostasis of transition metals is severely altered in AD, with extracellular pooling of metals such as Zn and Cu in amyloid, and intraneuronal accumulation of Fe within the AD brain. Abnormal Zn, Cu, and Fe levels have been observed in AD brains (Deibel, Ehmann, and Markesbery 1996). In addition, transition

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metals have been detected in amyloid deposits and transgenic mouse models (Lovell et al., 1998; Zhang et al., 2006).

The development of clinical symptoms of AD requires many decades for pathological changes to develop (McDade et al., 2018). However, during the early stages of the disease, the body's cellular defense mechanisms, including the DNA damage response, will activate and potentially stop the progression of AD (Jackson and Bartek 2009).

As seen in cancer, cellular response mechanisms involve multiple pathways. The role of one of these genes, TP53, is now being revealed as central to the cellular response to cancer and other diseases, including obesity and aging-associated neurodegenerative disorders (Labuschagne, Zani, and Vousden 2018). Given the implication of different mechanisms of cellular stress in AD pathology, understanding the role and regulation of TP53 and its protein product p53 in AD could unlock therapies to treat the disease in its early stages or facilitate AD diagnosis before the clinical manifestation of the disease.

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# p53 conformational changes: causes and consequences in AD

Since the discovery of p53 in 1979 (Lane and Crawford 1979; Linzer and Levine 1979), it has been accepted as a central tumor suppressor that has also been shown to be involved in diseases other than cancer, acting as a critical gatekeeper for cellular stress. p53 exerts its major functions as a homo-tetrameric transcriptional factor with a multidomain structure. By binding the p53-responsive elements located at target genes' promoters, p53 can activate the expression of multiple genes (Liu, Tavana, and Gu 2019).

The significant effect of p53 on tumors has been shown in numerous mouse experiments in which p53 loss-of-function predisposes cells to permanent damage and neoplastic transformation (Donehower et al., 1992; Olive et al., 2004). It has also been shown in p53 wild-type patients that many bear a dysfunctional p53 pathway due to various causes (Yue et al., 2017). Studies have also demonstrated that p53 functions as a central hub to deal with cellular oxidative stress. Besides DNA damage, p53 can respond to multiple upstream stress signals like oncogene activation, telomere erosion, ribosomal stress, and hypoxia (Horn and Vousden 2007). Once p53 is activated, it can regulate many cellular processes such as cell cycle arrest, DNA repair, apoptosis, ferroptosis, senescence, or autophagy to either promote cell survival or limit the malignant transformation of the cell.

In addition, p53 can also participate in the modulation of cell metabolism, pluripotency, epigenetic states, and aging (Kastenhuber and Lowe 2017). And, as p53 has so many diverse roles in maintaining cellular function, any change in the protein's functional activity will

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greatly affect its downstream processes (Vousden and Lane 2007).

The role of p53 stretches far beyond simply dealing with DNA damage. p53 should be regarded not as the 'guardian of the genome' but as a cellular stress guardian.

There are several cellular mechanisms controlling p53 function, and amongst them, p53 post-translational modifications (PTMs) represent the most widespread and effective type of regulation (Chen, Liu, and Tao 2020).

## Biochemical reactions resulting in post-translational modifications of the p53 linear sequence

The multi-modular structure of the p53 protein facilitates modifications such as phosphorylation, ubiquitination, acetylation, methylation, SUMOylation, neddylation, O-GlcNAcylation, ADP-ribosylation, hydroxylation, and β-hydroxybutyrylation (Chen, Liu, and Tao 2020) (Figure 1).

Phosphorylation of p53 occurs mainly at serine and threonine residues of the N-terminus and C-terminus. Interestingly, studies have reported that phosphorylation at Ser15 induced MDM2 dissociation with the p53 protein, resulting in the stabilization of the p53 complex (Shieh et al., 1997). Ser15 can be phosphorylated by the AMP-activated protein kinase (AMPK) pathway and mediates the metabolic cell cycle G1/S block (Jones et al., 2005). Phosphorylation of p53 at position 392 can be induced by DNA damage and plays a role in activating the DNA binding capacity of p53. Phosphorylation can affect the conformation of p53 by altering the formation of p53 tetramers, which affects their activity (Chène 2001).

Acetylation of eight lysine residues of p53 (K120, K164, K370, K372, K373, K381, K382, and K386) is induced by cytotoxic stimuli, resulting in the stabilization and activation of p53, with

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the recruitment of transcriptional activators in the promoter region of p53 downstream genes, and their activation (Brooks and Gu 2011a; Barlev et al., 2001). Acetylation of p53 is critical for its ability to inhibit the cell cycle progression of the G2 phase (Imbriano et al., 2005). Acetylation at the p53 C-terminal lysine residue also prevents the ubiquitination at the same lysine residue site induced by MDM2. This further stabilizes the tetramer, enhances the DNA binding ability of p53 to specific sequences, and promotes the recruitment of transcriptional activators (Itahana, Ke, and Zhang 2009). The mechanism of p53 transactivation is dependent on different promoters, with acetylation modification playing an important role in the selective regulation of p53 function, and the role of acetylation dependent on the cellular environment.

Methylation at lysine and arginine is an important reversible mechanism for the regulation of p53 function (Jansson et al., 2008). Lysine methylation in the nucleus after DNA damage: enhances the stability of p53 chromatin binding; increases recruitment of p53 in regulatory regions of p21 and other downstream genes; promotes transcriptional activation of p21 and other downstream genes (Chuikov et al., 2004). Arginine methylation also modulates the activity of p53 and is an important regulatory mechanism of the p53 response. When DNA is damaged, arginine methylation of p53 is extensively promoted (Jansson et al., 2008). Methylation by PRMT5 is also known to influence the activity of p53 oligomerization (Jansson et al., 2008).

Ubiquitination of MDM2 is an essential factor in maintaining p53 levels by promoting the polyubiquitination of p53 and its degradation by the proteasome pathway, inhibiting p53-mediated transactivation (Brooks and Gu 2011b; Haupt et al., 1997). The major ubiquitination sites of p53 mediated by MDM2 are the six lysines at the carboxy terminus (Rodriguez et al.,

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2000). The expression of MDM2 is also regulated by p53, indicating a negative feedback loop controlling p53 expression. Thus, an increase in p53 level can induce MDM2 expression, leading to a decrease in p53 expression and activity (Wu et al., 1993). Ubiquitination prevents p53 from binding to the downstream gene in the nucleus, leading to apoptosis and cell cycle arrest (Lee et al., 2008).

Different types of p53 ubiquitination have different effects on its function and affect p53 stabilization by regulating nuclear export and cytosolic localization. Ubiquitination also disrupts p53 from binding to the promoter of target genes as a transcription factor in the nucleus that results in apoptosis and cell cycle arrest (Lee and Gu 2009).

In addition to the main modifications described, there are other modifications of p53, such as SUMO-1 and SUMO-2/3-mediated SUMOylation (Wu and Cheng 2009) and neddylation (NEDD8) (Abida et al., 2007; Xirodimas et al., 2004).

These modification types have some common features: (i) Multiple sites: each modification type can occur on many different amino acids (ii) Multiple functions: the functions of the modifications are site-specific. (iii) Reversibility: for each modification, there are de-modification enzymes. (iv) Cross talk: modifications can influence the effects of modifications at other sites (Liu, Tavana, and Gu 2019).

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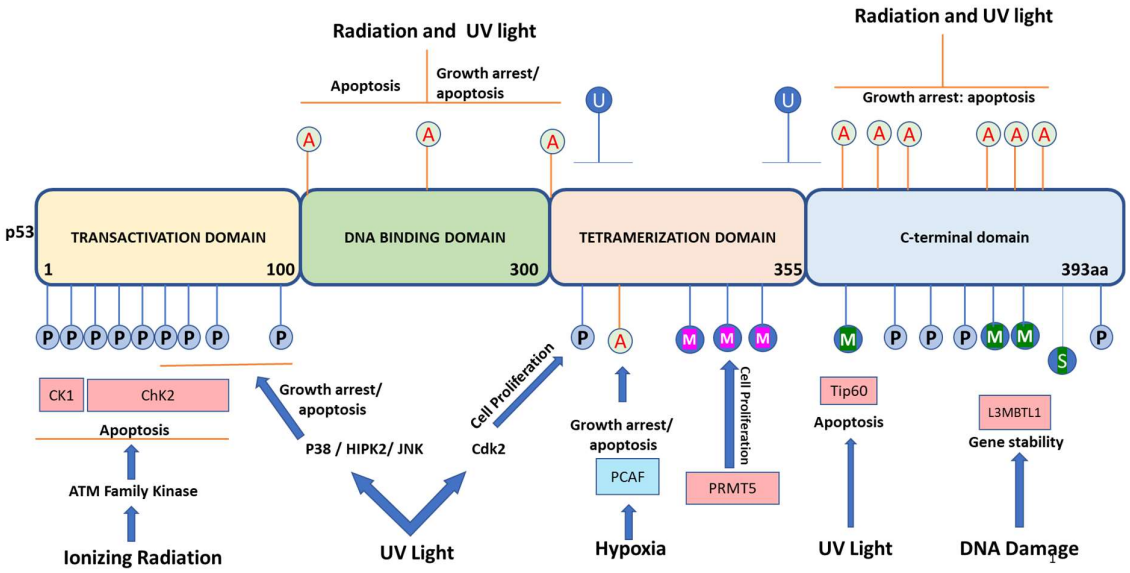


**Figure 1. Overview of p53 PTMs.** The major sites for p53 modifications (phosphorylation, ubiquitination, SUMOylation, neddylation, acetylation, methylation, O-GlcNAcylation, ADP-ribosylation, hydroxylation, and β-hydroxybutyrylation) are plotted. Different colors are used to differentiate the types of modification. Modified from Chen et al., (2020).

ROS and reactive nitrogen species (RNS) are by-products of aerobic metabolism. RNS trigger a signaling cascade that can be transduced through oxidation-reduction (redox)-based post-translational modifications (redox PTMs) of protein thiols. This redox signaling is essential for normal cellular physiology and coordinately regulates the function of redox-sensitive proteins (Metodiewa and Kośka 1999). It plays a particularly important role in the brain, which is a major producer of RNS. Aberrant redox PTMs of protein thiols can impair protein function and are associated with several diseases where redox PTMs, including S-nitrosylation, could be important factors in brain aging and in neurodegenerative disease (Finelli 2020). The

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mechanisms of these modifications include affecting cellular stress control proteins such as p53 stability and localization and even protein conformational changes (Buizza et al., 2012). In AD, an unfolded conformation of p53 has been identified and linked to AD pathology (Uberti et al., 2006; Buizza et al., 2012; Abate et al., 2020a).

In normal unstressed cells, p53 is kept at a low level through ubiquitination by its major negative regulators MDM2 and MDM4 (MDMX), which target TP53 for proteasomal degradation. MDM2/MDM4 also binds to transactivation domain 1 (TAD1) and inhibits the ability of TP53 to interact with transcriptional components, thus preventing transcription. TP53 PTMs modulate TP53 stability as well as its interactions with DNA, chromatin, and many cofactors that influence TP53-mediated transcription or repression of target genes. TP53 directly activates the transcription of several hundred genes through binding to response elements near promoters or in enhancers; some genes are also directly repressed (Riley et al., 2008).

## p53 function and the link to AD

p53 protein function has been shown to be closely associated with many cell stress control mechanisms and is proposed to be intrinsically involved in defense against neuronal degeneration. Such mechanisms include maintenance of redox homeostasis, inflammation, and redox control, along with regulation of the neuronal cell cycle and control of Aβ peptides (Lanni et al., 2007). Thus, deregulation of a central protein such as p53 could significantly contribute to neuronal dysfunction and play a critical role in neurodegeneration. Figure 2 summarizes the main factors that can impact p53 conformation and highlights within the current models of AD pathogenesis the main areas implicated.

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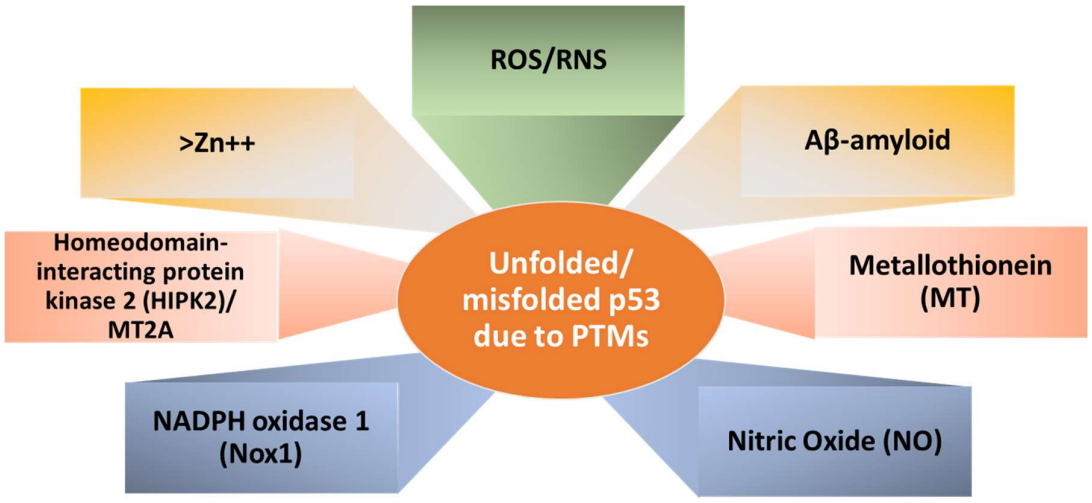


**Figure 2. Key factors that affect the generation of the conformational change of p53 to an unfolded conformation in the current model understanding of Alzheimer's disease.** Each box refers to a significant cellular factor linked to PTMs of the p53 protein.

p53 is involved in the regulation of oxidative stress, which, as discussed earlier, is the imbalance of ROS and the activity of antioxidant enzymes to maintain homeostasis. Uberti et al. (2006) implicated this as one of the earliest events in AD where evidence of oxidative stress has clearly been seen in post-mortem studies of AD brains along with evidence that oxidative stress can induce Tau hyper-phosphorylation (Zhao and Zhao 2013). Growing evidence has proposed oxidative stress to be a critical factor in both AD initiation and progression with a strong relationship to Aβ and Tau-induced neurotoxicity forming feedback loops to accelerate AD progression (Zhao and Zhao 2013). The Aβ-induced depletion of Homeodomain Interacting Protein Kinase 2 (HIPK2) along with the unfolded p53 may also contribute towards cellular dysfunction along with AD pathogenesis (Puca et al., 2008; Puca et al., 2010; Lanni et

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al., 2010). With further regards to oxidative stress, the free radical of nitric oxide (·NO) has been shown to be involved with PTMs of p53, with nitration of the protein found at tyrosine 327, which promotes p53 oligomerization and activation (Mocchegiani et al., 2004). Metallothionein is a small cysteine-rich protein thought to play a critical role in the cellular detoxification of inorganic species by sequestering metal ions that are present in elevated concentrations. Specifically, metallothionein isoforms (I+II and III) and interleukin-6 in the hippocampus of old rats, where their concomitant increments could lead to neurodegeneration (Mocchegiani et al., 2004). NADPH Oxidase 1 (Nox1) has been shown to be involved in p53 deacetylation and suppression of its transcriptional activity and apoptosis (Puca et al., 2010). Nox1 is activated in the hippocampus under Chronic Cerebral Hypoperfusion (CCH), causing oxidative stress and consequential hippocampal neuronal death, and cognitive impairment. This evidence implies that Nox1-mediated oxidative stress plays an important role in neuronal cell death and cognitive dysfunction in dementia (Choi et al., 2014).

Amyloid plaque accumulation in the AD brain has also been linked to p53 (LaFerla et al., 1996; Lapresa et al., 2019; Farmer et al., 2020; Abate et al., 2020a). This is not surprising due to the many functions of p53 in its role as a 'cellular guardian.' Any deregulation will affect many signaling pathways implicated in AD, with p53 at the crossroads of a complex network of stress response pathways (Stanga et al., 2010). With regards to the effect of amyloid on p53 conformation, it has been postulated that sublethal amounts of Aβ inhibit the Zyxin/HIPK2 signaling axis, upregulating MT2A resulting in chelated zinc atoms causing a change in p53 conformation (Abate et al., 2020b).

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HIPK2 modulation leads to the up-regulation of MT2A, which chelates Zn ions leading to p53 conformational change (Lanni, Nardinocchi, et al., 2010; Lanni et al., 2013). With Zn++ redox signaling and transition metals associated with control of the p53 pathway (Méplan, Richard, and Hainaut 2000), restoring wt-p53 activity in HIPK2 depleted cells can be accomplished by modulating metallothionein and zinc (Puca et al., 2009).

An inflammatory environment leading to immune cells migrating to the site of amyloid plaques in the AD brain has been reported in many studies and has been implicated as a potential third major AD pathology hypothesis (Kinney et al., 2018). The presence of a highly active immune response in the AD brain, such as the activation of the brain macrophages and other immune cells, has been shown to be involved with amyloid and Tau pathology, with microglia-related signaling mechanisms being implicated in AD. However, the exact mechanisms involved still need to be elucidated (Kinney et al., 2018).

Cell cycle deregulation has been implicated in AD as a greater number of dividing cells have been noted in AD brain vs. controls (Zhou and Jia 2010). The cell cycle hypothesis may explain the slow rate of atrophy during AD progression (Zhou and Jia 2010).

The areas discussed above are all regulated by p53, and deregulation with a subsequent loss of function due to a PTM-related conformational change to unfolded p53 could link p53 to the pathogenesis of AD. The impact of the unfolded conformational variant of p53 is summarized in Figure 3, where the implicated AD pathways are directly linked to the specific factors related to the conformational change in p53.

In addition, the functional p53 protein is critical to ensuring that aging cells are suitably regulated. With most of the research over the last 40 years completed on p53 and cancer (Feng, Lin, and Wu 2011; Wu and Prives 2017) and more recently in other disease areas,

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murine studies have been extensively used to study aging with a number focusing on isoforms of p53. Findings indicate that over-expression of terminally truncated p53 (as p44) protein is potentially linked to aging and aggregation of Tau in NFT's (Pehar et al., 2010; 2014). These aged mice also had synaptic deficits and a similar decline in cognitive function to AD, and interestingly a significant increase in Tau phosphorylation suggesting a Tau/p53 link in AD pathogenesis (Pehar et al., 2010; 2014). Within the human AD brain, studies have implicated changes in expression of p53-beta and delta 133 p53 isoforms in the pathology of AD brains compared to controls (Turnquist et al., 2016).

# p53 signaling pathways in the pathogenesis of AD

As discussed in the previous section, the main regulation of p53 is by protein-protein interaction, and many post-translational modifications (PTM) can regulate p53 activity and could be a critical point of dysregulation during the development of AD (Chen, Liu, and Tao 2020).

During cellular stress, the p53 response can recruit a number of key signaling proteins, including cell cycle checkpoint inhibitors and others (Shieh et al., 1997; Budanov, 2014).

The normal cellular defense activity of p53 is an action to repair the cellular damage or to lead the cell to apoptosis; these are critical processes during cellular aging (Budanov 2014). Interestingly, the expression of ataxia-telangiectasia mutated (ATM) was shown to be increased in a range of mild to moderate to severe AD patients compared to controls and

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correlated with plaque density and Braak stages (Katsel et al., 2013). These data strongly indicate an active p53 mediated DNA damage response is being carried out in AD.

The physiological function of p53 is to respond to oxidative stress, as shown by the activation of antioxidant genes and their associated signaling pathways. These pathways include Manganese Super Oxide Dismutase (MnSOD) and the TP53 induced glycolysis and apoptosis regulator (TIGAR) (Sablina et al., 2005; Budanov 2014). AD studies have shown an impaired antioxidant response, with low MnSOD activity in an AD mouse model (Sompol et al., 2008). However, there are several conflicting studies showing expression of TIGAR is inversely correlated with the severity of AD, although in mild dementia, downregulation of TIGAR was noted in the superior temporal cortex (Sablina et al., 2005; Katsel et al., 2013). *In vitro* experiments on BACE, the rate-limiting enzyme linked to the generation of Aβ, transcription is repressed by p53, thus affecting potential Aβ accumulation. In oxidative damage exposure, the levels of carboxy-terminus Hsc70-interacting protein (CHIP) decreased, which is important in BACE1 degradation and APP stability. CHIP functions as a molecular co-chaperone and ubiquitin E3 ligase in controlling the degradation of numerous chaperone-bound proteins. p53 overexpression inhibits BACE1 expression contributing to AD pathogenesis. In summary, BACE1 is a downstream gene of p53, which is downregulated by CHIP-mediated p53 activation and CHIP-mediated degradation in both transcriptional and post-transcriptional aspects (Singh and Pati 2015).

The mTOR signaling pathway has been implicated many times in p53 activity, and interestingly, this pathway has been shown to be activated in early AD before dementia (Li et al., 2005; Feng et al., 2005; Yates et al., 2013; Sidorova-Darmos, Sommer, and Eubanks 2018). An AD transgenic mice study has indicated that a build-up of Aβ increased the function of the

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mTOR signaling pathway resulting in a negative feedback loop inhibiting autophagy and thus the clearance of Aβ peptides. This evidence suggests that activation of the mTOR pathway may be caused by dysregulated p53 but requires further studies to confirm this hypothesis.

Sirtuin 1 (SIRT1) is a member of the sirtuin family, which regulates metabolic activity in mammalian cells through deacetylation/acetylation. Sirtuins are NAD+-reliant deacetylases. Sirtuin deficiency disrupts mitophagy in numerous diseases, including AD (Sidorova-Darmos, Sommer, and Eubanks 2018). Nuclear p53 governs PINK1, a crucial protein involved in the control of mitophagy, by repressing its promoter activity, protein, and mRNA levels of SIRT1 triggers expression of neurons in AD (Sidorova-Darmos, Sommer, and Eubanks 2018).

A recent publication has shown another potential p53 regulated pathway involved in AD development, involving an additional member of the sirtuin family; the downregulation of sirtuin 6 (SIRT6). The SIRT6 pathway promotes cell longevity by controlling inflammation, genome integrity, energy metabolism, and DNA repair. With a reduction in SIRT6 protein levels shown in human AD brain samples (Jung et al., 2016). The control mechanism was investigated in a neuronal cell line. Aβ 42 post-transcriptionally reduced p53 protein levels resulting in a decreased binding of p53 to the SIRT6 promoter, downregulating the p53 gene related to aging (Jung et al., 2016).

SIRT6 protein expression is decreased in the brains of 5XFAD AD model mice and AD patients. Upregulation of p53 protein by Nutlin-3 averts SIRT6 decline and DNA impairment induced by Aβ (Jung et al., 2016).

One of the stimulatory/inhibitory functions of SIRT1 is around its connection with p53. In postnatal cortical neurons, p53 apoptotic function needs PUMA transcriptional activation.

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PUMA with Bax-mediated permeabilization of the mitochondrial outer membrane leads to cytochrome C release and caspase activation, which triggers cell death. SIRT1 inhibited apoptosis by deacetylating p53 in senescence models (Talebi et al., 2021).

Interestingly, a recent study showed the exposure of neurons to fragments 25-35 of Aβ peptide activates Cdk5, which promotes p53 phosphorylation and stabilization (Lapresa et al., 2019). Mitochondrial dysfunction and neuronal apoptosis were prevented when Cdk5 and p53 functions were inhibited. Thus, Cdk5 is activated by fragments 25-35 of Aβ inducing p53 phosphorylation and stabilization, and this could be an attractive therapeutic target against an Aβ induced neurodegeneration feedback loop (Lapresa et al., 2019).

The first evidence of a conformational change of p53 in AD was shown by Uberti et al. (2002). They showed that an AD conformational change in p53 occurs in skin fibroblasts isolated from AD patients. Upon the exposure of these skin fibroblasts to hydrogen peroxide ($H_2O_2$), there is no activation of p53 dependent cell cycle regulators p21, GADD45, and BAX1 genes (Uberti et al., 2006). This impairment resulted in an accelerated re-entry and diminished $H_2O_2$-related apoptosis when compared to control fibroblasts. However, this mechanism was not initially shown in neurons but clearly showed DNA damage repair mechanism impairment in peripheral cells. The lack of p53 activity was caused by a conformational change in the tertiary structure of the protein; the result is inactivity on the promoters of p21 and GADD45. It was later confirmed that the p53 gene was not mutated, and only the protein conformation had been changed (Cordani et al., 2016). The authors proposed this effect was AD specific as *in vitro* models expressing the APP-751 protein and exposure of fibroblasts from control subjects to Aβ 1-40 and 1-42 induced the same conformational change, and this was noted in AD compared to other dementias and cancer. The proposed mechanism was linked to the Zyxin

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pathway with Aβ peptides being implicated in deregulating the Zyxin protein leading to proteasomal degradation and downregulation of HIPK2 and the up-regulation of MT2A, which chelates Zn ions leading to p53 conformational change. This is reversible with the addition of Zn ions increasing the sensitivity of cells to acute cytotoxic damage and levels of growth-associated protein 43 (GAP-43) (Lanni et al., 2010; 2013). The conformationally changed variant, an unfolded p53, also increases amyloid precursor protein (APP) metabolism and Aβ load, with a high level of unfolded p53 expression resulting from loss of p53 proapoptotic activity and a decrease in mRNA and protein levels of GAP-43 (Buizza et al., 2012). As a control, treating HEK cells with an Aβ sequestering antibody resulted in the partial prevention of the p53 conformational change indicating a direct link to Aβ (Uberti et al., 2007). Further studies using Immortalized lymphocytes derived from AD patients were shown to induce the p53 conformational change due to nitration of certain tyrosine residues (Buizza et al., 2012). Studies utilizing lymphocytes from AD patients showed an increase in CD44 when increased p53 is also observed, which may also link the lymphocytic control pathways. The evidence supports the hypothesis that the conformational change of p53 could be a significant contributor to the dysregulation of the mTOR AD signaling pathway previously discussed in this review.

The main AD signaling pathways implicated with p53 conformational change are summarized in Figure 3.

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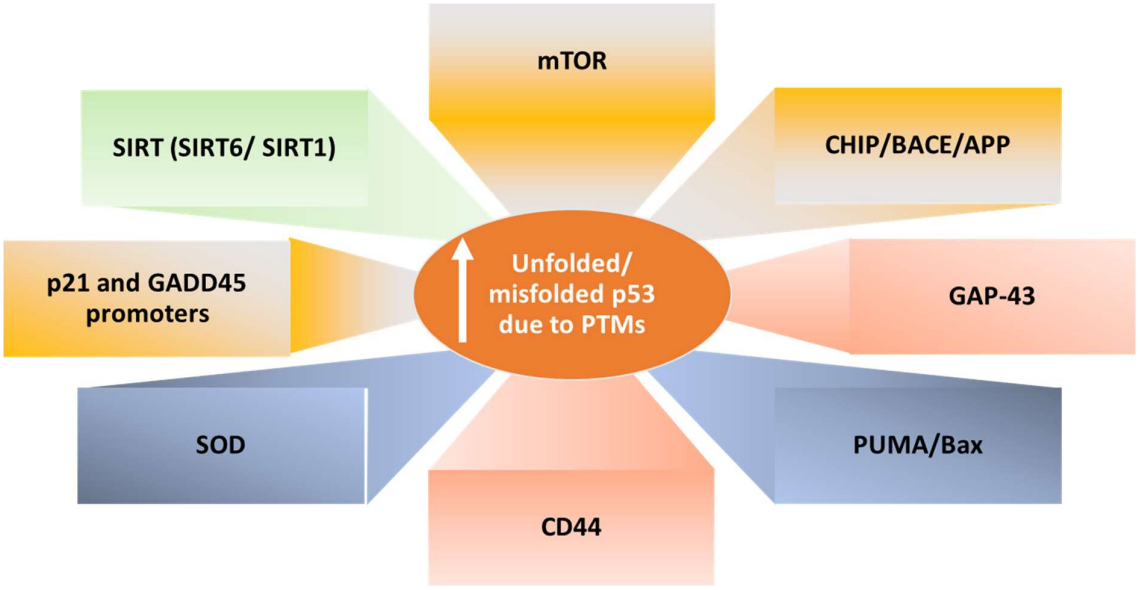


**Figure 3. Summary of the main signaling pathways that are affected by the PTM-induced conformational changes causing unfolded p53.**

The relationship between Tau, Aβ, and p53 has been explored in many studies and has been considered as a potential therapeutic target for AD (Jazvinšćak Jembrek et al., 2018). The recent paper from Sola et al. (2020) focused on the effect of Tau on p53 function during the DNA damage response, studying acute DNA damage in Neuroblastoma cells after Tau depletion. Under these conditions, altered p53 stability and activity resulted in reduced cell death, linking the interaction between p53 and its E3 ubiquitin ligase MDM2 (Sola et al., 2020). Farmer et al. (2020) further investigated the potential role of Tau and p53 in AD in relation to an impaired DNA damage response. Farmer et al. (2020) demonstrated that p53 forms oligomers and fibrils in the human AD brain but not in human control brains. The p53 protein was shown to interact with Tau oligomers in AD brains and can be recapitulated by *in vitro* Tau oligomer treatment in C57BL/6 mouse primary neurons. p53 oligomers also colocalize, potentially seeding endogenous p53 in primary neurons in the presence of DNA

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damage, and phosphorylated p53 was shown to be blocked outside the nucleus. p53-mediated DNA damage responders were also shown to be decreased in the AD brains, with control brains showing a normal DNA damage response mechanism. This could indicate that a loss of nuclear p53 function in AD may be due to p53 aggregation and/or interactions with Tau oligomers. A linear p53 hypothesis is proposed in Figure 4.

It was also shown that when Aβ is present at nanomolar levels, throughout the inhibition of HIPK2, it induces the expression of metallothionein 2A, which, having $Zn^{++}$-chelating activity, sequesters metals from the DNA-binding-domain of p53 and induces conformational changes in p53, inhibiting its activity. This directly links to the study by Farmer et al. (2020), where the interaction of p-Tau and p-p53, and the localization of p-p53 outside the nucleus, was observed.

A possible link between the p53 conformational variant and Aβ by HIPK2–p53 signaling has also been confirmed by an *in vitro* study (Jazvinšćak Jembrek et al., 2018). Furthermore, a study using a low-grade pro-oxidant environment activated p53 intracellular pathways, affecting its tertiary structure and inducing conformational changes and the loss of its activity (Abate et al., 2020).

Since p53 regulates a heterogeneous repertoire of biological functions, including neuronal outgrowth and protection of neuronal connectivity, regulation of innate immunity, and redox homeostasis (Y. Li et al., 2019), these findings reinforce the hypothesis that loss of function of p53 due to its conformational change in the early stages of AD may contribute to several

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associated pathologies including synapse dysfunction, inflammation, and oxidative stress. Therefore, the p53 conformational variant could be a biomarker of early AD pathological events. These will include the accumulation of Aß, redox deregulation, and immune activation leading to oxidative stress and chronic inflammation. The link of p53 and its conformational variant, caused by PTMs and linked to Tau and Aß, is summarized in Figure 4.

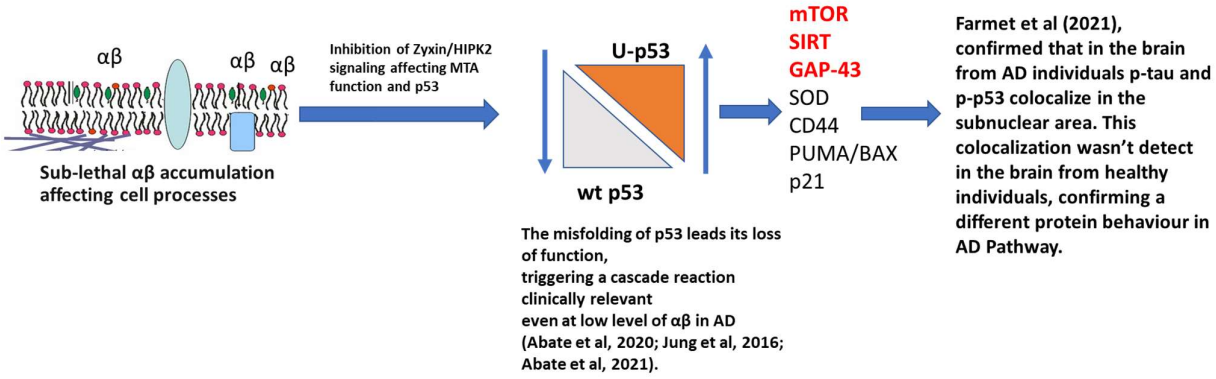


**Figure 4 p53 hypothesis in AD.** Sub-lethal accumulation of Aß affects cellular pathways leading to the misfolding of p53 and its loss-of-function, triggering a clinically relevant cascade reaction. In the AD brain, phosphorylated p53 (p-p53) and p53 aggregates are overexpressed compared to the healthy individuals (controls), confirming a different protein behavior than the WT isoform. mTOR, SIRT, and GAP-43 highlighted in red are postulated to be the major pathways involved.

## The conformational variant of p53 detected in AD

The importance of the unfolded conformational variant of p53 caused by PTMs has been investigated in several studies comparing AD patients to controls, and a significantly higher expression of the conformational variant of p53 was detected in peripheral blood mononuclear cells (PBMCs) from AD patients (Uberti et al., 2008; Lanni, Racchi, et al., 2010; Stanga et al., 2012). Interestingly, unfolded p53 was independent of APOE ε4 status (Abate et

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al., 2020a). p53 levels were significantly increased in PBMCs from preclinical patients with mild cognitive impairment (MCI) when compared to controls. This led to a study showing increased levels of unfolded p53 protein were highly predictive of the conversion from amnestic MCI to AD dementia (Uberti et al., 2008; Lanni et al., 2010; Stanga et al., 2012).

As discussed earlier, the expression of the unfolded conformational variant of p53 was investigated in other dementias, including Parkinson's disease, where the data indicated that p53 is differentially expressed when compared to AD patients, thus suggesting specificity for AD (Uberti et al., 2008).

To further investigate the relationship of unfolded p53 isoforms in AD, Memo et al. (2018) developed a monoclonal antibody (mAb) 2D3A8 to be highly specific for the p53 conformational variant and targeted to potential nitrosylation sites. On detailed characterization, the antibody was shown to recognize a linear epitope between the p53 DNA binding domain and the conjunction region with the tetramerization domain. The authors propose this region becomes exposed due to redox post-translational modifications owing to the pro-oxidant environment in AD.

The recent study by Abate et al. (2021) further evaluated the presence of the conformational variant of p53 using the mAb 2D3A8. Three hundred seventy-five plasma samples from two longitudinal AD studies were used in conjunction with a machine learning approach to create an algorithm to predict AD likelihood risk. The concentration of p53 conformational variant detected by 2D3A8 and quantified by an in-house plasma ELISA method, Mini-Mental State Examination (MMSE), and levels of apolipoprotein E epsilon-4 (APOE4) were considered. The study created an AD likelihood risk and showed a robust 86.67% agreement with clinical diagnosis. Interestingly the algorithm showed a highly significant area under the curve (AUC

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= 0.92) for amnestic Mild Cognitive Impairment (aMCI) patients who will develop AD, which would indicate the potential of this as a prognostic test for the development of AD.

In a further study, the conformational variant of p53, detected by 2D3A8 and then quantified by mass spectroscopy (identified as U-p53$^{AZ}$), was further validated as a potential prognostic biomarker in AD in a set of plasma samples from the longitudinal and retrospective biobank, AIBL (Piccirella et al., 2021). In detail, 482 individuals (515 total samples) up to 144 months after baseline and at different stages of cognitive decline due to AD were analyzed. The U-p53$^{AZ}$ was shown to have a high prognostic value, predicting the progression to AD from preclinical or prodromal AD with a significant AUC> 0.90, showing prognostic validity more than six years prior to signs of clinical symptoms. Additionally, the prognostic performance of this conformational variant of p53 was also higher than other main risk factors alone or in combination with amyloid status. U-p53$^{AZ}$ was shown to have high diagnostic performance to segregate cognitively normal individuals from those with AD (AUC values >0.90).

# Conclusion

The exact pathology of AD is controversial and remains to be fully elucidated. The validity of the amyloid hypothesis is still being challenged due to the failure of several high-profile drug trials. A significant amount of evidence supports the potential role of p53 in AD pathogenesis, particularly due to the protein's functional dysregulation and involvement in many AD pathways. Post-translational modifications are an important part of the normal regulation of p53 cellular function, and the presence of a conformational change in p53 induced by redox dysfunction in AD, leading to a loss of function in many cellular response pathways, is an indication of a central role in AD. Further studies on the conformational unfolding of p53

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showed that the conformational change of the protein impacts on its role, directly increasing or decreasing the activation of specific pathways involved in AD. Recent studies have also shown that p53 is expressed in the AD brain in a phosphorylated isoform in correlation with p-Tau (Farmer et al., 2020). This study supports the effect of PTMs on p53 conformation and its role in AD. Recent studies (Piccirella et al., 2021; Abate et al., 2021) also showed the expression of a conformational variant of p53 in the plasma samples of asymptomatic and AD prodromal individuals confirming the role of the conformational variant of p53 in AD.

Further studies will be necessary to further elucidate the mechanism of actions for the specific PTMs of p53 and their involvement in AD pathways.

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