Accelerated neurodegeneration through chaperone-mediated oligomerization of tau

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Aggregation of tau protein in the brain is associated with a class of neurodegenerative diseases known as tauopathies. Fk506 binding protein 51 kDa (FKBP51, encoded by FKBP5) forms a mature chaperone complex with Hsp90 that prevents tau degradation. In this study, we have shown that tau levels are reduced throughout the brains of Fkbp5–/– mice. Recombinant FKBP51 and Hsp90 synergized to block tau clearance through the proteasome, resulting in tau oligomerization. Overexpression of FKBP51 in a tau transgenic mouse model revealed that FKBP51 preserved the species of tau that have been linked to Alzheimer’s disease (AD) pathogenesis, blocked amyloid formation, and decreased tangle load in the brain. Alterations in tau turnover and aggregate structure corresponded with enhanced neurotoxicity in mice. In human brains, FKBP51 levels increased relative to age and AD, corresponding with demethylation of the regulatory regions in the FKB5 gene. We also found that higher FKBP51 levels were associated with AD progression. Our data support a model in which age-associated increases in FKBP51 levels and its interaction with Hsp90 promote neurotoxic tau accumulation. Strategies aimed at attenuating FKBP51 levels or its interaction with Hsp90 have the potential to be therapeutically relevant for AD and other tauopathies.

Introduction

Neurofibrillary tangles, hallmarks of a class of neurodegenerative diseases termed tauopathies, are comprised of microtubule-associated tau protein aggregates that persist in the brain even after neuron death (1). In the most common tauopathy, Alzheimer’s disease (AD), the presence of these tangles correlates with pathology, development and disease progression. However, levels of neuronal death in AD far exceed what is expected based on the observed numbers of tau tangles (2), suggesting that a different mechanism is primarily responsible for neurodegeneration. One possible explanation for this discrepancy is that soluble tau species not visible by standard histological methods are the main cause of neurotoxicity in tauopathies (3). In fact, neurons may form tangles as a protective mechanism to sequester these more toxic soluble intermediates and convert them into a less harmful form (4, 5). These soluble tau species may also be able to spread from neuron to neuron in a fashion similar to that of prions, further establishing their role in perpetuating pathogenicity (6–8). Mutations in MAPT, the gene encoding tau, can also lead to the production of soluble tau conformers with pathogenic potential, leading to rarer tauopathies, such as progressive supranuclear palsy and frontotemporal dementia with parkinsonism linked to chromosome 17 (9–11). Thus, identifying the mechanisms that contribute to the production of soluble tau intermediates could be paramount for developing therapeutic approaches to treat AD and other tauopathies.

The variability in age of onset for AD and other tauopathies, even those caused by bona fide hereditary mutations, indicates that other environmental- and experience-based genetic modifiers are involved in the production of pathogenic tau species (12, 13). One group of age-affected genes that can also modulate tau proteostasis is the molecular chaperone family, which includes heat shock proteins (Hsps), proteins whose expression increases under conditions of stress (14). Levels of Hsps diminish with age, and roles for these proteins in neurodegenerative disease in relation to the age of onset have been proposed in several models (15). In particular, the Hsp90 system is of interest as a potential tau “toxifier” (16, 17).

We and others have shown that inhibiting Hsp90 can promote tau clearance, suggesting that Hsp90 is acting to protect tau in neurons for unknown reasons (16, 18, 19). Hsp90 can even enhance tau amyloid formation in vitro, a unique function in comparison with those of other chaperones. Both of these functions of Hsp90 are in contrast to the effects of other chaperones that have been shown to enhance tau clearance and subvert its amyloidogenesis (20). Though several cochaperones of Hsp90 can influence tau stability in cells, this has not been well studied in the brain. For instance, the Fk506 binding protein 51 kDa (FKBP51, encoded by FKBP5) is known to work with Hsp90 to stabilize tau and regulate tau phosphorylation (17), but the mechanisms and pathological relevance for both of these processes are unknown. FKBP51 possesses properties that are similar to those of the cis-trans peptidyl-prolyl isomerase Pin1, which has been reported on extensively (21–23); however, much less is known about the role of FKBP51 in the brain despite its genetic association to human...
psychiatric diseases (24). FKBP51 is a profolding Hsp90 cochaperone that forms a complex with Hsp90 via the tetratricopeptide (TPR) domain (25, 26), a region also found on other Hsp90 cochaperones, including the carboxyl terminus of Hsp70-interacting protein (CHIP; also known as STUB1) and FK506 binding protein 52 (FKBP52; also known as FKBP4) (27, 28). However, the TPR cochaperone with the highest affinity for Hsp90 is FKBP51 (29). Here we investigate how the FKBP51/Hsp90 complex prevents clearance of tau, the impact it has on tau pathogenesis, and whether it plays a role in disease onset due to age-associated changes in TPR protein levels in the human brain. Our findings suggest that environmental or experiential-based epigenetic changes that accelerate upregulation of FKBP51 over time could be a major contributor to the onset and pathogenicity of tauopathies and, in particular, AD.

Results
FKBP51 depletion reduces tau levels in brain. Previous work showed that FKBP51 depletion reduced tau levels in cell models of tauopathy (17). However, it was not clear whether this was relevant in vivo. To address this issue, brain tissue from Fkbp5-/- mice was collected and subjected to both immunohistochemical and biochemical analyses. Mice were generated as previously described (30, 31). Staining of Fkbp5-/- mice revealed a significant reduction in tau levels throughout the brain (Figure 1, A–C). Similarly, Western blot analyses of brain homogenates showed similar reductions in several tau epitopes, including a significant reduction in total tau (as assessed with the Tau 12 [amino acids 2–18] and H150 [tau N terminus] antibodies) and phosphorylated tau (as assessed with the CP13 [pS202] and pT231 [PHF6] tau antibodies) (Figure 1, D and E). We then performed fluorescent analyses to confirm

Figure 1
Tau expression is decreased in the Fkbp5-/- mice and associates with FKBP51 in the AD brain. Representative images of brain tissue from (A) wild-type and (B) Fkbp5-/- mice stained for pT231 tau and corresponding sections without primary (no primary). Scale bar: 2,000 μm. (C) Quantification of pT231 stain (SEM). **P = 0.0097. (D) Representative Western blot of brain homogenates from Fkbp5-/- mice probed for CP13 (pS202/pT205), Tau 12 (total tau), pT231 tau, H150 (total tau), and GAPDH. (E) Quantification of duplicate Western blots (± SEM). *P < 0.05 for CP13, Tau 12 (total tau), and H150 (total tau); ***P < 0.001 for pT231. (F) Fluorescent micrograph of human cortex stained with FKBP51, PHF1 (pS396/pS404), and Neurotrace (neuronal nuclei) antibodies (original magnification, x60). Scale bar: 50 μm.
that FKBP51 and tau were colocalized in neurons in human brain. Immunostaining with FKBP51 antibody and PHF1 (pS396/S404 tau) antibody that stains human AD brain well, combined with a neuronal marker, showed colocalization of neuronal tau pathology and FKBP51 in AD cortex (Figure 1F).

FKBP51 works with Hsp90 to synergistically block the 20S proteasome from degrading tau. Previously we reported that FKBP51 coordinated with Hsp90 to promote the accumulation of nonubiquitinated tau in the presence of a proteasome inhibitor (17). This suggested that FKBP51 may be playing a role in ubiquitin-independent tau degradation via the 20S proteasome, a process shown to be a major route of tau clearance (32, 33). The 20S proteasome serves as the catalytic core for the 26S proteasome (34), and 20S proteasomes can be about 3- to 4-times more abundant in cells than 26S proteasomes (35). In addition, FKBP51 was previously found to slow the degradation of tau by chymotrypsin, the major protease of the 20S proteasome (17). To determine unequivocally whether the FKBP51/Hsp90 complex could alter the kinetics of clearing tau by the 20S proteasome, we used a reconstituted in vitro assay, as previously described (32). Indeed, tau was rapidly degraded by the 20S proteasome, but this was partially blocked by FKBP51 alone (Figure 2A). When Hsp90 and FKBP51 were combined, tau degradation by 20S proteasome was completely blocked (Figure 2, B and C), whereas Hsp90 alone had effects on tau similar to those of FKBP51 alone. The effects of FKBP51 and Hsp90 together were significantly greater than the additive effects of each individually, suggesting true synergy (Figure 2D). Changes in degradation were not attributed to nonspecific alteration of the 20S proteasome, as the 20S reporter substrate was not affected by the addition of FKBP51 (Figure 2E).

FKBP51 coordinates with Hsp90 to produce nonamyloid, Thioflavin T–negative tau oligomers. The synergistic effects of Hsp90 and FKBP51 directly on tau proteasomal degradation suggested that these 2 chaperones somehow worked together to regulate tau conformation and possibly its aggregation kinetics. To get a general idea about how FKBP51 and Hsp90 regulated tau structure, circular dichroism (CD) analyses were performed with tau in combination with FKBP51, Hsp90, or both proteins together. As previously shown, tau is an intrinsically disordered protein, and its CD spectra have a minima at 200 nm (36). Indeed, the tau produced in our lab reproduced these results, showing a spectrum from 190 to 260 nm, typical for a highly disordered protein such as tau (Figure 3A). The secondary structures of FKBP51 and Hsp90 were also determined by CD. Consistent with previous reports, FKBP51 and Hsp90 showed substantial ordered secondary structure (28, 37). The PONDR-FIT algorithm, one of the most accurate disorder predictors (38), confirmed the CD data, indicating that tau is almost completely disordered, while FKBP51 and Hsp90 are largely ordered (Supplemental Figure 1; supplemental material available online with this article; doi:10.1172/JCI69003DS1). Next, CD spectra were collected for tau in the presence of FKBP51 alone, Hsp90 alone, or both chaperones combined (Figure 3B). The dashed lines in Figure 3B show the predicted spectra of these protein mixtures if no structural change were
to occur. These calculated spectral plots were obtained by summing the spectra from each of the individual measurements taken for tau, FKBP51, or Hsp90; however, the observed CD spectra (Figure 3B, shown as solid lines) were in contrast to the calculated spectra, suggesting that structural changes were occurring when these proteins were combined. While FKBP51 alone (3:10 ratio of FKBP51 to tau) had no effect on the secondary structure of tau (Figure 3B), Hsp90 (2:10 ratio of Hsp90 to tau) did alter tau structure. Interestingly, when FKBP51, tau, and Hsp90 were incubated together at a respective 3:10:2 ratio, tau showed a unique structural change unlike that...
Figure 4
FKBP51 overexpression by viral vector in rTg4510 mice preserves tau. (A) GFP or FKBP51 was overexpressed in the HPC of rTg4510 mice using an AAV9 vector. Scale bar: 2,000 μm; 50 μm (inset). Original magnification, ×63 (inset). (B) Total tau (± SEM) was measured using ImageJ in neurons expressing FKBP51 or GFP. At least 15 images were taken per animal. ***P < 0.0001. (C) Representative images FKBP51- and GFP-expressing neurons (arrows) (original magnification, ×63) using antibodies toward GFP (green), FKBP51 (green), total Tau (red), and NeuN (blue). Scale bar: 50 μm. (D) Tissue from infected mice was stained for pT231 tau. pT231-positive area (± SEM) was measured per CA3. *P = 0.0463. Representative sections are shown. Scale bar: 2,000 μm; 200 μm (inset). Original magnification, ×10 (inset). (E) T22 oligomeric tau (± SEM) was measured in the CA3 region of FKBP51- and GFP-overexpressing mice. *P = 0.0176. Scale bar: 2,000 μm; 200 μm (inset). Original magnification, ×10 (inset). (F) Representative Western blot of brain homogenate from FKBP51- or GFP AAV–injected mice immunoblotted with FKBP51, pT231, H150 (total tau), and GAPDH. T22 antibody on complimentary dot blot. 4163

after incubation with Hsp90 or FKBP51 alone, suggesting that FKBP51 only affected tau structure when Hsp90 was present. It is important to note that these spectra changes are still present when the calculated spectra are scaled to the global minima (Supplementary Figure 2A). Formal calculations of predicted secondary structure were performed using DichroWeb (39), and these are included in the supplement (Supplemental Figure 2B).

Since altering the structure of aggregation-prone proteins is known to contribute to their pathogenic assembly into fibrillar structures (40–43), we speculated that Hsp90 alone and Hsp90 with FKBP51 could alter tau fibril assembly. We used dynamic light scattering (DLS) to assess how the Hsp90/FKBP51 complex was regulating the kinetics of global tau aggregation (44). For these studies, tau aggregation was induced with heparin sulfate in the presence of Hsp90 or FKBP51 alone and in the presence of Hsp90 and FKBP51 together. A 50:1 tau/chaperone ratio was used to minimize any contributions of the chaperones themselves to the signal produced. Tau combined with these Hsp90/FKBP51 mixtures was allowed to polymerize over a period of 6 days, and particle size distributions were determined with DLS throughout the duration of the experiment. Similar to the results using CD spectroscopy, FKBP51 alone did little to impact tau aggregation kinetics; both tau alone and tau with FKBP51 reached the half-maximal size by 2.3 ± 0.3 days (Figure 3C). The rate of aggregation was also unaffected (k_app = 1.7 ± 0.3 days). Hsp90 alone actually accelerated tau aggregation (time for half-maximal size, t_half = 1.5 ± 0.0 days; apparent rate, k_app = 3.3 ± 0.0 days) consistent with previous findings (20). Surprisingly, though, FKBP51 was able to slow the Hsp90-induced tau aggregation (time for half-maximal size, t_half = 2.1 ± 0.1 days; apparent rate, k_app = 2.0 ± 0.5 days) (Figure 3C), further supporting our hypothesis that FKBP51 uses Hsp90 to regulate tau biology and specifically promote its pathogenesis. Incubating these tau aggregates with Thioflavin T, a dye that specifically recognizes β-pleated sheet amyloid structure, showed that Hsp90 promoted τ β-sheet aggregates, but FKBP51 prevented this (Figure 3D). Therefore, FKBP51 prevents tau from adopting β-sheet structure in the presence of Hsp90, promoting the production of amorphous tau aggregates. These results were confirmed using atomic force microscopy (Figure 3E). To determine whether recombinant tau was indeed forming oligomeric structures, tau was incubated with FKBP51 or Hsp90 alone or with FKBP51 and Hsp90 together with heparin, as described for the DLS exper-

Figure 5
FKBP51 selectively promotes tau neurotoxicity. (A) Sections from FKBP51- and GFP AAV–injected mice were stained with Gallyas silver stain and positive area (± SEM) per CA3 was measured. *P = 0.0297. Representative images of each group are shown. Scale bar: 2,000 μm; 200 μm (inset). (B) Neurons stained by cresyl violet and NeuN were counted (± SEM) by stereology in the CA3 region. **P = 0.0003. (C) Representative images are shown. Scale bar: 500 μm; 50 μm. Original magnification, ×20 (inset). (D) Volume of CA3 (± SEM) in injected transgenic mice was evaluated by stereology.
The mixtures of these proteins were then analyzed by dot blot with the anti-tau oligomer–specific antibody, T22 (3). Indeed, T22-positive tau was only observed in the presence of FKBP51. The production of this oligomeric species was enhanced, even though total tau levels were the same, when FKBP51 and Hsp90 were incubated together with tau (Figure 3F). Hsp90 alone produced no T22 immunoreactivity. In this way, FKBP51 causes Hsp90 to alter its structural influence on tau, leading to the formation of nonamyloid tau aggregates and oligomeric tau species, which have both been implicated as the primary toxic tau species that facilitate neurodegeneration (4, 5, 45, 46). Based on these findings, we speculated that FKBP51 overexpression in the brain may drive tau toxicity. Therefore, we sought to evaluate the effects of FKBP51 in vivo.

**In vivo, FKBP51 overexpression preserves tau species, including oligomers.** The hippocampi (HPC) of a cohort of 4-month-old rTg4510 transgenic mice, which express human P301L mutant human tau, were injected bilaterally with AAV9 particles expressing either FKBP51 (n = 5) or GFP (n = 5). These mice have abundant Hsp90 levels and low basal FKBP51 levels at 6 months of age, and they are the best model to recapitulate human tau pathobiology (5, 45, 47–49). Therefore, 2 months following these injections, 6-month-old mice were harvested and examined histologically, as previously described (47). FKBP51 expression was observed in the HPC; however, the AAV did not transduce all neurons (Figure 4A). Due to this typical limitation of AAV use, a fluorescent masking technique previously developed by our lab was used to evaluate the levels of tau in neurons express-
in vitro data suggested that FKBP51 also prevented amyloid formation. To further confirm this result, primary neurons from tau-expressing pups were transduced with FKBP51 AAV9 particles and allowed to express for 14 days. On the 14th day, an MTS assay was performed to measure toxicity. Significant toxicity was found in tau-expressing neurons transduced with FKBP51 AAV9 compared with GFP AAV9–transduced controls (Supplemental Figure 3). Additionally, Neuro2A cells, a mouse neuroblastoma line, were transfected with tau, FKBP51, or the combination for 48 hours. MTS assays performed at the end of the 48 hours revealed significant cell toxicity in the neuronal line, but only in the cells transfected with tau and FKBP51 together (Supplemental Figure 3).

**FKBP51 prevents tangle formation but enhances tau neurotoxicity.** Our in vitro data suggested that FKBP51 also prevented amyloid formation. The rTg4510 mouse is one of the only transgenic models to produce Gallyas silver-positive tangles that are a traditional pathological hallmark for AD and other tauopathies (47). Gallyas silver stains the same structure as Thioflavin T (50), but Gallyas lacks the fluorescent properties of Thioflavin T. Thus, the previous characterization of Gallyas silver in this model combined with its lack of fluorescence in the GFP spectrum made it the ideal stain to assess β-sheet structure in these studies. Silver-positive tau was significantly reduced in the CA3 region of mice injected with FKBP51 AAV compared with that in those injected with GFP AAV (Figure 5A). Thus, FKBP51 overexpression in neurons promoted the accumulation of nonamyloid oligomeric tau species, similar to what was observed in vitro.

The toxicity of these T22-positive/silver-negative tau species produced by FKBP51 overexpression were then directly evaluated using unbiased stereology. Indeed, in rTg4510 mice expressing FKBP51, there was a significant decrease in the number of neurons in the CA3 hippocampal region compared with that in mice injected with GFP AAV (Figure 5, B and C). This neuron loss did not significantly alter CA3 volume (Figure 5D). Since this was the first time that FKBP51 and tau have been shown to be neurotoxic, we performed in vitro experiments to confirm this result. Primary neurons from tau-expressing pups were transduced with FKBP51 AAV9 particles and allowed to express for 14 days. On the 14th day, an MTS assay was performed to measure toxicity. Significant toxicity was found in tau-expressing neurons transduced with FKBP51 AAV9 compared with GFP AAV9–transduced controls (Supplemental Figure 3). Additionally, Neuro2A cells, a mouse neuroblastoma line, were transfected with tau, FKBP51, or the combination for 48 hours. MTS assays performed at the end of the 48 hours revealed significant cell toxicity in the neuronal line, but only in the cells transfected with tau and FKBP51 together (Supplemental Figure 3).

**Figure 7**

**FKBP51 DNA demethylation with aging.** Average FKB5 methylation analysis of human DNA samples from the PCG of individuals aged 21, 36, 42, 74, 85, 95 years were performed on multiple CpG sites in (A) intron 7, (B) promoter, and (C) intron 2. Significant demethylation in intron 7 was found at P1_S1_Pos2 (r = 0.0012, r = 0.6506). P1_S2_Pos1 (P = 0.0007, r = 0.9586). P1_S2_Pos2 (P = 0.0039, r = 0.9002). P1_S2_Pos3 (P = 0.0240, r = 0.9002), and P1_S2_Pos3 (P = 0.0240, r = 0.7583). Methylation was significantly decreased in the promoter at GRE_S1_G1 (P = 0.0327, r = 0.7199) and GRE3_S1_G3 (P = 0.0216, r = 0.7698).
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FKBP5 DNA methylation decreases with aging, a mechanism for its increased expression. It was recently shown that decreases in FKBP5 DNA methylation increase the expression of FKBP51 (54). To see whether this was the mechanism leading to the significantly increased FKBP51 expression found in the aged brain, methylation analysis using pyrosequencing was performed on DNA from PCG brain tissue of aging nondemented samples ($n=6$) on multiple CpG sites in functional glucocorticoid response elements in intron 7, intron 2, and the promoter (Figure 7) of FKBP5 (54). While intron 2 showed no significant linear decrease in methylation with aging, intron 7 and the promoter had 7 CpG sites for which DNA methylation was significantly decreased with aging. Significant demethylation in intron 7 was found at 5 CpG positions (Figure 7A). Methylation was significantly decreased in the promoter in 2 locations (Figure 7B). These age-associated decreases in DNA methylation in specific regulatory elements could explain why FKBP51 expression is progressively increased with age (Figure 6E).

FKBP51 expression is further increased in the AD brain, perhaps through altered FKBP5 DNA methylation. Given the role of FKBP51 on tau accumulation and its pathogenicity, we examined whether FKBP51 levels were elevated further in AD brain compared with those in normal age-matched controls. FKBP51 mRNA expression was measured to compete with FKBP51 for binding to Hsp90 (29), had decreasing trends (Figure 6A), but were not significantly decreased with age in any region measured (Supplemental Figure 4, C and D). Additionally, we found that increasing FKBP51 expression levels were significantly correlated with age (Figure 6B). This was consistent with FKBP51 expression data observed in human prefrontal cortex through the BrainCloud program (13). Similar results were observed at the protein level in the PCG by Western blot (Figure 6, C and D). Thus, the aging brain promotes an environment in which FKBP51 can dominate the Hsp90 machine, potentially leading to tau pathogenesis.

Based on these data, we hypothesized that in human tauopathies, increasing levels of FKBP51 could allow it to usurp Hsp90 from other cochaperones with lower affinity, causing it to produce toxic tau oligomers, rather than sequester tau into more benign amyloid fibrils (29). Indeed, in cells expressing constant levels of CHIP, an Hsp90 cochaperone that can facilitate tau clearance through the proteasome (52, 53), increasing FKBP51 levels were able to displace CHIP from Hsp90 and preserve tau levels (Figure 6E). When taken together, these data suggest that elevated FKBP51 can overtake the Hsp90 machinery in the aging brain and alter tau structure in a way that facilitates its neurotoxic accumulation.
Hsp90 levels appeared unchanged, while the levels of FKBP52 and CHIP trended toward a decrease in AD samples. In addition, those samples with higher tau levels, regardless of disease diagnosis, also had overall higher FKBP51 levels, further supporting a link between FKBP51 and tau accumulation.

Based on these results, we speculated that increasing FKBP51 levels might correlate with disease progression or disease onset. To assess this possibility, correlation analyses were performed based on Braak staging. We chose Braak staging as the most unbiased measure for disease progression. Indeed, increased FKBP51 levels were significantly correlated with increased Braak staging, suggesting that FKBP51 may have a role in AD progression (Figure 8E). Furthermore, when the aged samples were evaluated alone, the significant increase in Braak staging with FKBP51 expression was maintained (Supplemental Figure 5). In this way, FKBP51 may play a role in accelerating disease onset and progression by facilitating the production and accumulation of toxic tau, leading to AD and expediting its pathogenesis.

To evaluate the role of AD on FKBP5 DNA methylation, the same pyrosequencing analyses as described above were performed on brain tissue from the medial temporal gyrus of AD samples (n = 10) and those of aged-matched controls (n = 5). There was a significant decrease in methylation at 3 CpG sites; 1 in intron 7 (Figure 9A) and 2 in the promoter (Figure 9B). There was only one position in which DNA methylation was increased in the AD population (Figure 9C). There was also a decreasing trend of overall FKBP5 methylation in intron 7 as well as the promoter areas at every tested CpG of AD diagnosed samples compared with non-demented age-matched controls (Supplemental Figure 6).

**Discussions**

We have found that a chaperone, FKBP51, can work with Hsp90 to produce oligomeric tau in the brain, facilitating its neurotoxicity. FKBP51 overexpression enhanced neuronal loss in the rTg4510 tau transgenic mouse model, suggesting that FKBP51 coordinates with Hsp90 to prevent tau clearance and facilitate its toxicity. This provides further evidence that other forms of tau, besides those that are pathologically visible in postmortem tissue like tangles, are highly neurotoxic (5, 45, 55, 56). Thus, factors that facilitate FKBP51 expression could promote an environment in the aging brain that supports tau pathogenesis. We show that FKBP51 methylation is reduced both with age and in disease, which could be one factor leading to increased FKBP51 expression (54, 57, 58). FKBP5 methylation and FKBP51 expression appear to be inversely proportional over time. Therefore, it is certainly conceivable that age could combine with other factors, such as stress or genetic variation, to increase FKBP51 expression to detrimentally high levels. We found that FKBP51 levels were further enhanced in the AD brain compared with those in age-matched controls, possibly resulting from FKBP5 demethylation in these functional regulatory elements, as shown previously (54). In fact, the progressive increases in FKBP51 levels correlated with Braak
staging in the entire sample population as well as the nondemented aging subgroup population alone. Additionally, since the FKBP5 gene is responsive to stress hormone (54), the levels of which increase in AD (59), FKBP51 could be exploited as a novel biomarker to compliment other such molecules for evaluating AD risk.

Based on these findings, we predict that people predisposed to increased levels of FKBP51 could display a higher risk for AD onset and possibly a more rapid progression of the disease. On the other hand, it is possible that the pathogenic processes that manifest in AD facilitate increased FKBP51 expression as well, which could also explain why FKBP51 levels are further increased in individuals with AD compared with those in age-matched controls. Either scenario would likely create a vicious cycle in the brain that contributes to disease progression.

At the molecular level, these studies show that FKBP51 can prevent tau clearance through the 20S proteasome without directly impairing 20S proteosomal activity. This is one of the few examples in which chaperones can coordinately interact with a substrate, in this case tau, to block its degradation. This type of control of tau degradation may be unique to tau because of its high level of disorder (60). Since the 20S proteasome typically proteolyzes unfolded proteins (35), it is possible that FKBP51 and Hsp90 bind tau, giving it more order and preventing its indiscriminate degradation through the 20S proteasome. But this appears to come at a price: FKBP51 and Hsp90 also promote a structure for tau that produces toxicity, supporting the idea of the need for balance in the proteostasis network for healthy neuronal aging and function (61). When chaperone levels are too high or too low, some substrates that are particularly difficult to handle can begin to accumulate and cause disease. These substrates can then have detrimental consequences to the proteostasis system and general cellular health (62–65).

Perhaps the most intriguing result from these studies is the discovery that a chaperone can mediate the formation of tau oligomers. These data also show that subverting amyloid formation could produce a deleterious consequence. Recent work has begun to question the role of amyloids in AD (66). While amyloid pathology (tangles, plaques, Lewy bodies, etc.) is the hallmark of many neurodegenerative diseases, these types of pathologies do not correlate well with neuronal loss or dysfunction in animal models of disease. For example, in the rTg4510 tau transgenic mice, neurons with tau tangle structures are longer lived (4, 67). This same model displays cognitive recovery when tau is suppressed, despite continued tangle formation (5, 46). Moreover, strategies that deplete soluble intermediate tau species without impacting tangle pathology have been very successful at improving cognitive impairment and neuronal loss in transgenic tau models (5, 46, 68). The current evidence suggests that sequestration of misfolded or abnormally folded proteins into amyloid β-sheet structures may be more beneficial to neurons than allowing these intermediates to traverse the intracellular milieu (69–71). FKBP51 not only interferes with tau degradation and promotes tau oligomer formation, but FKBP51 also impairs tau’s ability to be sequestered into less toxic tangles, facilitating tau toxicity. This is why strategies aimed at depleting FKBP51 or inhibiting its association with tau or Hsp90 may be highly effective for tau-based therapeutics.

Overall, this study shows that a significant increase in FKBP51 levels in the aged and AD brain could promote an environment that preserves toxic tau oligomers. We show that FKBP51 coordinates with the Hsp90 machinery to alter tau structure, block its degradation, and promote abnormal oligomer formation. This strongly implicates FKBP51 as a therapeutic target for AD and other tauopathies. These data ultimately shed light on the nature of tau accumulation and support a new body of research, suggesting that chaperone proteins impede rather than aid in the pathogenesis of these diseases.

Methods

A full description of all materials and methods is available in the Supplemental Methods. See complete unedited blots in the supplemental material. Data for the microarray have been deposited in the Gene Expression Omnibus (GEO) database, with accession number GSE11882.

Statistics. To compare 2 groups, a Student’s t test was used. Groups larger than 2 were evaluated using a 1-way ANOVA test. Linear regression was used to show the relationship between 2 variables. Values below 0.05 were considered significant.

Study approval. All studies were carried out following the guidelines set by the University of South Florida’s Institutional Animal Care and Use Committee in accordance with the Association for Assessment and Accreditation of Laboratory Animal Care International regulations.

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