Published in final edited form as:

Brain Res. 2011 April 22; 1386: 191–199. doi:10.1016/j.brainres.2011.02.052.

β -amyloid triggers ALS-associated TDP-43 pathology in AD models

Alexander M. Herman^b, Preeti J. Khandelwal^a, Brenna B. Stanczyk^a, G. William Rebeck^a, and Charbel E-H Moussa^{a,b}

^aDepartment of Neuroscience Georgetown University Medical Center. Washington D.C. U.S.A. 20007

^bDepartment of Biochemistry Molecular and Cell Biology Georgetown University Medical Center. Washington D.C. U.S.A. 20007.

Abstract

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease associated with loss of motor neurons in the brain and spinal cord. ALS is occasionally diagnosed with frontotemporal lobar dementia with ubiquitin positive inclusions (FTLD-U). Alzheimer's disease (AD) is the most common type of age-associated dementia. Abnormal levels of aggregated Tar-DNA binding protein-43 (TDP-43) are detected in the majority of patients with ALS, FTLD and AD. We observed a significant increase (200%) in the levels of TDP-43 in cortical autopsies of late stage AD patients. Lentiviral expression of A β ₁₋₄₂ in the rat motor cortex led to an increase in TDP-43 pathology, including up-regulation of the mature ~44kDa protein, identical to the pathological changes seen in AD. Furthermore, expression of A β ₁₋₄₂ was associated with TDP-43 phosphorylation and accumulation in the cytosol. Clearance of A β with parkin prevented TDP-43 pathology. TDP-43 modifications were also observed in 3xTransgenic AD (3xTg-AD) compared to wild type mice, but these changes were attenuated in parkin-injected hippocampi, even in the presence of Tau pathology, suggesting that TDP-43 pathology is triggered by A β , independent of Tau. Increased levels of casein kinase (CK1 and CK2), which are associated with TDP-43 phosphorylation, were also observed in A β ₁₋₄₂ expressing brains. These data indicate an overlap in TDP-43 pathology between AD and ALS-FTLD and suggest that A β triggers modifications of TDP-43.

1-Introduction

The number of neurodegenerative diseases associated with pathological aggregates of transactivation response element (TAR)-DNA-binding protein 43 (TDP-43) has increased in the last decade. TDP-43 is a 414-amino acid protein with a theoretical molecular mass of 44.74 kDa. The expressed protein contains two highly conserved RNA recognition motifs (RRM1 and RRM2), as well as a glycine-rich C-terminal, which mediates protein-protein binding (Ardley et al., 2001; Morett and Bork, 1999). Full-length TDP-43 is localized predominantly to the nucleus, with small amounts of cytosolic presence under normal

Author to whom all correspondence is to be addressed: Charbel E-H. Moussa, MB. Ph.D. Department of Neuroscience. Department of Biochemistry, Molecular & Cell Biology. Georgetown University School of Medicine. 3970 Reservoir Rd, NW, TRB, Room WP26B. Washington DC 20057. Tel: 202-687-7328. Fax: 202-687-0617. cem46@georgetown.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

conditions (Buratti et al., 2005; Buratti and Baralle, 2008; Wang et al., 2004; Winton et al., 2008). TDP-43 pathology both in the brain and spinal cord is characterized by decreased solubility, ubiquitination, hyper-phosphorylation and cleavage of TDP-43 into 25 and 35kDa fragments, as well as cellular translocation from nuclear to cytosolic compartments (Amador-Ortiz et al., 2007; Geser et al., 2008; Hasegawa et al., 2007; Mackenzie et al., 2007; Neumann et al., 2006; Neumann et al., 2007a; Neumann et al., 2007b; Tan et al., 2007; Zhang et al., 2007). TDP-43 was identified in the inclusions of frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U) and Amyotrophic Lateral Sclerosis (ALS) (Neumann et al., 2006). FTLD is one of the major causes of dementia in young adults (Ratnavalli et al., 2002; Snowden et al., 2002) and comprises a group of heterogeneous neurodegenerative disorders that are occasionally associated with motor neuron disease (MND) (Bennett et al., 1999; Neary et al., 1990; Neary et al., 2000; Tofaris et al., 2001).

TDP-43 is a major constituent of inclusions in motor and non-motor neurons in ALS and FTLD-MND (Arai et al., 2006; Neumann et al., 2007a; Tan et al., 2007). ALS is a neurodegenerative disorder that affects both upper and lower motor neurons, leading to progressive paralysis and death (Pasinelli and Brown, 2006). Only ~20% of ALS cases are familial associated with missense mutation in Cu/Zn superoxide dismutase gene (SOD1) (Gros-Louis et al., 2006; Rosen, 1993). Most ALS cases are sporadic with 50% of patients display coincident deterioration of both motor and cognitive function (Morita et al., 2006; Talbot and Ansorge, 2006) and 20% develop clinical features suggestive of FTLD (Lomen-Hoerth et al., 2002; Lomen-Hoerth et al., 2003). Pathologically, ALS patients have TDP-43 accumulation in motor neurons (Ayala et al., 2005; Neumann et al., 2006) and Tau-negative ubiquitin inclusions identical to those of FTLD-U patients (Forman et al., 2006). Although no TDP-43 mutations have been associated with FTLD-U, several mutations (Q331K, M337V, G294A, A90V) have been identified in MND/ALS (Gitcho et al., 2008; Sreedharan et al., 2008). TDP-43 is also altered in AD, the most common cause of dementia in the elderly. A large number (75%) of AD cases show TDP-43 pathology (Amador-Ortiz et al., 2007). Lewy body disorders also demonstrate TDP-43 pathology in AD with LBD, PD and PD with dementia (Nakashima-Yasuda et al., 2007). Co-localization between TDP-43 and NFTs and TDP-43 and α -Synuclein in dystrophic neurites were also identified, despite studies showing lack of co-existence between TDP-43 and Tau pathologies (Arai et al., 2006; Nakashima-Yasuda et al., 2007; Neumann et al., 2007b).

The pathology of AD is characterized by intraneuronal deposition of hyper-phosphorylated Tau as well as extracellular β -amyloid ($A\beta$) plaques (Hardy and Selkoe, 2002). $A\beta$ is produced intracellularly via the endosomal system and secretory pathways that mediate the processing of amyloid precursor protein (Haass et al., 1992; Koo and Squazzo, 1994). $A\beta_{1-40}$ and $A\beta_{1-42}$ are produced intracellularly (Cook et al., 1997; Greenfield et al., 1999; Lee et al., 1998; Skovronsky et al., 1998; Xu et al., 1997), and accumulate in the brain of individuals with AD [Wilson et al., 1999; Gouras et al., 2000]. Both intracellular and extracellular oligomeric $A\beta$ have been implicated in AD pathology, but intracellular oligomeric species may be formed first to act in the earlier stages of disease (Gouras et al., 2000; Wilson et al., 1999). Immunocytochemical studies on AD, Down's syndrome (DS) and APP transgenic mouse brains reveal abundant intraneuronal $A\beta$ (Hanihara et al., 1995; Hof et al., 1992; Hyun et al., 2002; Ito et al., 2008; Lee et al., 2001; Morales et al., 2002; Murray et al., 2005; Ros et al., 2008; Sanchez et al., 2002; van de Warrenburg et al., 2001).

Since neurodegenerative diseases often involve the accumulation of several misfolded proteins, we sought to ask whether specific pathogenic proteins cause the accumulation of TDP-43. We generated animal models using lentiviral gene delivery of $A\beta_{1-42}$ (Burns et al., 2009) and α -Synuclein (Khandelwal et al., 2010) to mimic the pathology of AD and PD,

respectively. We also used 3xTg-AD mice, which display A β and hyper-phosphorylation of Tau. We compared TDP-43 pathology in animal models to autopsies of human patients diagnosed with late stage AD. Finally, we analyzed the effects of the E3-ubiquitin ligase parkin to reduce A β and α -Synuclein levels in gene transfer models and 3xTg-AD mice. These studies allow us to better understand the overlapping biochemical pathways underlying neurodegeneration at the interface between different neurological diseases, including FTLD, ALS, PD and AD.

2-Results

TPD-43 pathology is increased in AD brains

To examine TDP-43 pathology in the human brain autopsies, we analyzed motor cortex from late stage AD (N=12) patients and age-matched (N=7) control subjects (John Hopkins University Medical Institution) using Western blots. Analysis of lysates from AD patients (Fig. 1A, top blot) showed an increase in full-length APP and C-terminal fragments (CTFs) compared to age-matched controls. Probing these blots with anti-TDP-43 (2E2-D3) antibody (Fig. 1A, upper middle blot) revealed an increase in the level of full length ~44 kDa TDP-43. A smaller TDP-43 band (~15kDa) was also detected, perhaps due to TDP-43 cleavage. A protein smear was also observed between 62-98Kd, suggesting aggregation of TDP-43 in AD patients compared to control subjects. An increase in phosphorylated TDP-43 (pTDP-43) was also observed in AD (Fig. 1A lower middle blot) compared to control brains, which included both full length and cleaved TDP-43. Densitometric analysis (Fig. 1B) of blots revealed a significant (~200%) increase in the level of the ~44kDa band of TDP-43 ($P<0.05$, Neuman Keuls with multiple comparison) in AD patients compared to control subjects, suggesting that TDP-43 pathology co-exists with A β accumulation in AD.

Intracellular A β_{1-42} induces TPD-43 up-regulation, cleavage, and aggregation in gene transfer animal models

To test whether TDP-43 pathology is associated with either A β or α -Synuclein expression, we used lentiviral gene transfer animal models to express intracellular A β_{1-42} (N=8) or α -Synuclein (N=8) and examined for changes in endogenous TDP-43 processing. All lentiviral clones, including parkin, A β_{1-42} and α -Synuclein, were injected with lentiviral LacZ to control for viral m.o.i. (2×10^{10}) used. Lentiviral A β_{1-42} was injected into the motor cortex in the presence or absence of lentiviral parkin and lentiviral α -Synuclein was injected into the striatum and animals were sacrificed 4 weeks post-injection. The brains were dissected out and analyzed by Western blots. As expected, animals injected with lentiviral A β_{1-42} had a significant (200%, N=8, $P<0.05$) increase in the ~44kDa band of TDP-43 (Fig. 2A) in the rat primary motor cortex and the appearance of bands at ~28kDa and 62-98kDa compared to lentiviral LacZ, lentiviral parkin or lentiviral α -Synuclein expressing rats. We previously showed that co-expression of lentiviral A β_{1-42} and lentiviral parkin leads to clearance of intracellular A β_{1-42} (Burns et al., 2009). Injection with lentiviral parkin led to a significant increase (50%) in parkin levels in injected hemispheres compared to endogenous levels. Lentiviral parkin expression mediated the clearance of A β_{1-42} (lower blot) and prevented the changes in TDP-43 in the rat brain (Fig. 2A). Expression of lentiviral A β_{1-42} also resulted in phosphorylation (asterisks) of TDP-43 (Fig. 2B) and the appearance of a cleavage band ~28Kda and another unknown smaller ~15kDa band compared to lentiviral LacZ or lentiviral α -Synuclein injected rats. To examine the levels of kinases that are associated with TDP-43 phosphorylation, we examined the expression levels of casein kinase 1 (CK1) and 2 (CK2). The levels of CK1 and CK2 (Fig. 2B) were increased in A β_{1-42} compared to lentiviral LacZ, parkin and α -Synuclein injected brains, and parkin clearance of A β_{1-42} prevented the increase in CK1 and CK2 levels, suggesting that changes in kinase activity

may alter TDP-43 post-translational modification. These data suggest that intracellular A β ₁₋₄₂ increases TDP-43 levels, processing and aggregation.

To test the effects of A β on TDP-43 pathology over longer periods of time, we tested TDP-43 modification in 3xTg-AD mice (that express the APP_{sw}, PS1 and Tau_{P301L}) 3 months post-injection with lentiviral parkin into the right hippocampus (ipsilateral) and lentiviral LacZ into the left (contralateral) hippocampus. Lentiviral parkin led to a significant (50%) increase in parkin expression levels in the ipsilateral compared to the contralateral hemispheres of 3xTg-AD mice. Accumulation and cleavage of TDP-43 (Fig. 2C) were decreased in parkin-injected hemispheres compared to contralateral LacZ-injected hemispheres in 3xTg-AD mice. To further test the effects of A β expression in 3xTg-AD mice on TDP-43 pathology, we compared these transgenic mice to wild type controls (Fig. 2D). Western blot analysis showed 50% ($P < 0.05$) increase in mature (44kDa) TDP-43 and the appearance of 35kDa bands, suggesting cleavage of TDP-43 in 3xTg-AD mice compared to wild type control. Lentiviral parkin expression did not cause any changes to GSK-3 β activity (Fig. 2E) nor to the hyper-phosphorylation levels of Tau, suggesting that β -amyloid triggers TDP-43 modification independent of Tau.

Intracellular A β ₁₋₄₂ increases cytosolic accumulation of TDP-43

We performed an independent method to investigate changes in TDP-43 processing using immunohistochemical (IHC) staining of the motor cortex. Immunoreactivity of TDP-43 was predominantly visible within the DAPI stained nuclei (Fig. 3A) in the motor cortex of control (LacZ injected) rats. Injection of lentiviral LacZ+Lentiviral A β ₁₋₄₂ resulted in cytosolic accumulation of TDP-43 (Fig. 3B), and higher magnification images (Fig. 3C&D) revealed TDP-43 accumulation in the cytosol. These data suggest that A β ₁₋₄₂ can affect the modification of TDP-43 and accumulation in the cytosol. To further examine the effects of A β ₁₋₄₂ on TDP-43, we co-stained sections of motor cortex from lentiviral LacZ+lentiviral-A β ₁₋₄₂ injected rat brains. As expected we observed A β ₁₋₄₂ expression (Fig. 3E) and co-localization with TDP-43 (Fig. 3F&G), suggesting that cells that express A β ₁₋₄₂ also up-regulate the endogenous levels and cytosolic accumulation of TDP-43.

3-Discussion

This work shows the effects of amyloid proteins involved in the pathogenesis of a number of neurodegenerative diseases on the modification of TDP-43. An increase in TDP-43 protein level was observed in autopsies of AD patients and animal model expressing intraneuronal A β . Expression of intraneuronal A β ₁₋₄₂ was also associated with cytosolic TDP-43 accumulation, in agreement with previously reported data that show TDP-43 accumulation in the cytosol under abnormal conditions (Buratti et al., 2005; Buratti and Baralle, 2008; Caccamo et al., 2009; Wang et al., 2004; Wegorzewska et al., 2009; Winton et al., 2008; Zhang et al., 2009). TDP-43 phosphorylation, which we detected in AD brains and A β expressing animals, is also a prominent change in ALS and FTL-D (Hasegawa et al., 2008). These data suggest that intracellular A β triggers TDP-43 pathology, which was identified in the inclusions of FTL-D and ALS (Amador-Ortiz et al., 2007; Geser et al., 2008; Hasegawa et al., 2007; Mackenzie et al., 2007; Neumann et al., 2006; Neumann et al., 2007a; Neumann et al., 2007b; Tan et al., 2007; Zhang et al., 2007)). FTL-D is one of the major causes of dementia in young adults (Ratnavalli et al., 2002; Snowden et al., 2002) and is associated with ALS (Bennett et al., 1999; Neary et al., 1990; Neary et al., 2000; Tofaris et al., 2001). This type of dementia may be symptomatically related to AD-dementia and the coincident cognitive decline reported in ALS patients (Morita et al., 2006; Talbot and Ansorge, 2006). Most ALS cases are sporadic with 50% of patients display coincident deterioration of both motor and cognitive function (Morita et al., 2006; Talbot and Ansorge, 2006) and 20% develop clinical symptoms similar to FTL-D (Lomen-Hoerth et al., 2002;

Lomen-Hoerth et al., 2003). Furthermore, our data are consistent with recent findings, which showed that an increase in TDP-43 pathology is linked to accumulation of A β oligomers in 3xTg-AD mice and A β clearance with immunization reverses TDP-43 pathology without affecting Tau levels (Caccamo et al., 2010). Although animal models that express A β are different than AD brains, the effects observed in transgenic and gene transfer animals expressing A β are comparable to results obtained from AD brains, suggesting that A β may be a driving force of TDP-43 pathology.

The TDP-43 pathology associated with FTLN-ALS is a non-Tauopathy in which neuronal and glial inclusions are negative for Tau and α -Synuclein (Forman et al., 2006; Neumann et al., 2007a). Our animal models expressing intraneuronal A β ₁₋₄₂ or α -Synuclein show an increase in Tau phosphorylation, but lentiviral parkin expression reverses Tau modification in these animals (Khandelwal et al., 2010; Rebeck et al., 2010). However, Tau changes did not correlate with changes to TDP-43 in these animal models. Furthermore, lentiviral parkin did not change the levels of P301L Tau in the 3xTg-AD mice but reduced A β accumulation and TDP-43 levels, further suggesting that TDP-43 pathology is Tau-independent, consistent with other studies (Caccamo et al., 2010; Neumann et al., 2007a; Neumann et al., 2007b). Expression of lentiviral A β ₁₋₄₂ increased the levels of casein kinases associated with TDP-43 phosphorylation (Hasegawa et al., 2008), suggesting that intraneuronal β -amyloid accumulation activates kinases known to increase protein phosphorylation (Khandelwal et al., 2010; Rebeck et al., 2010). TDP-43 pathology has not been identified in primary Tauopathies (Davidson et al., 2007), but Tau pathology associated with AD is observed with TDP-43 pathology (Amador-Ortiz et al., 2007). Co-localization between TDP-43 and neurofibrillary tangles (NFTs) and TDP-43 in dystrophic neurites were identified, although other studies show lack of co-existence between TDP-43 and Tau pathologies (Arai et al., 2006; Nakashima-Yasuda et al., 2007; Neumann et al., 2007b). Taken together, these data are consistent with previous findings that show TDP-43 pathology in most (75%) AD cases (Amador-Ortiz et al., 2007). Lewy body disorders also display TDP-43 pathology in 30% AD with LBD, and 19% PD with dementia (Nakashima-Yasuda et al., 2007). However, the absence of α -Synuclein aggregation in these studies is notable and may be sufficient to explain the lack of α -Synuclein effects on TDP-43 pathology. β -amyloid induced TDP-43 pathology, particularly in brain regions involved in cognitive function, may be associated with dementia in neurodegenerative diseases.

These studies showed the effects of pathogenic accumulation of β -amyloid on FTLN/ALS-associated TDP-43 pathology, independent of Tau modification. TDP-43 pathology in animal models was similar to autopsies of human patients diagnosed with late stage AD, suggesting an overlap of biochemical pathways underlying neurodegeneration at the interface between different neurological diseases, including ALS, FTLN-U and AD. Our data suggest that A β triggers modifications of TDP-43 leading to its accumulation. More work is required to delineate the mechanisms of how A β ₁₋₄₂ increases TDP-43 pathology, particularly in cortical motor neurons in the brain and spinal cord, thus mimicking TDP-43-associated ALS pathology. We need to better understand the susceptibility of neurons in different brain regions to the accumulation of A β or TDP-43 and how the accumulation of one pathogenic protein affects the pathology of the other. Future studies that determine the mutual effects of TDP-43 and A β and the susceptibility of different brain regions to the pathogenic changes of these proteins may help understand the onset of dementia and cognitive deterioration in several neurodegenerative diseases.

4- Methods and Materials

Human brain autopsies

Autopsies of human motor cortex were collected 2-4 hours postmortem from late stage AD (N=12) patients and age-matched (N=7) control subjects at John Hopkins University Medical Institution. The average ages of these groups are 75 years old. The ages of AD patients were 65 to 83 years. Control subjects were 64 to 81 years old.

Stereotaxic injection

Lentiviral constructs were used to generate the animal models. Stereotaxic surgery was performed to inject the lentiviral constructs encoding LacZ, parkin, or α -Synuclein into the rat striatum (Khandelwal et al., 2010) and LacZ, $A\beta_{1-42}$, parkin, parkin+ $A\beta_{1-42}$ into the primary motor cortex (Burns et al., 2009) of male Sprague-Dawley rats weighing between 170-220g. Lentiviral parkin was also injected into the hippocampus of 3xTg-AD mice. All animals were anesthetized (50mg/kg body weight) with a cocktail of Ketamine and Xylazine (50:8). The stereotaxic coordinates were according to Paxinos and Watson brain atlas. Lentiviral stocks were injected through a Microsyringe pump controller (Micro4) using total pump (World Precision Instruments, Inc) delivery of 6 μ l at a rate of 0.2 μ l/min. Animals were injected into one side of the brain with a lentiviral-LacZ vector at 2×10^{10} m.o.i; or with 1×10^{10} m.o.i lentiviral- $A\beta_{1-42}$ + 1×10^{10} m.o.i lentiviral-LacZ; or 1×10^{10} m.o.i lentiviral- $A\beta_{1-42}$ + 1×10^{10} m.o.i lentiviral-parkin; and 1×10^{10} m.o.i lentiviral-Synuclein + 1×10^{10} m.o.i lentiviral-LacZ, and 1×10^{10} m.o.i lentiviral-parkin + 1×10^{10} m.o.i lentiviral-LacZ. Lentiviral gene transfer rats were sacrificed 4 weeks post-injection and 3xTg-AD mice were sacrificed 3 months post-injection with lentiviral parkin.

Western blot analysis

Brain tissues were homogenized in $1 \times$ STEN buffer (50 mM Tris (pH 7.6), 150 mM NaCl, 2 mM EDTA, 0.2 % NP-40, 0.2 % BSA, 20 mM PMSF and protease cocktail inhibitor), centrifuged at $10,000 \times g$ for 20 min at 4°C and the supernatant containing the soluble fraction of proteins were collected. The supernatant were analyzed on SDS NuPAGE 4-12% Bis-Tris gel (Invitrogen, Inc). Protein estimation was performed using BioRad protein assay (BioRad Laboratories Inc, Hercules, CA). Human anti- $A\beta_{1-42}$ was immunoprobed with 1:1000 mouse monoclonal (Zymed) antibody and parkin (1:1000) with rabbit polyclonal (PRK8) antibody (Upstate). Total TDP-43 was probed either with (1:1000) rabbit polyclonal antibody generated against N-terminal 260 amino acids of the full length protein (ProteinTech) or (1:1000) mouse monoclonal (2E2-D3) TDP-43 (Abnova). Phosphorylated TDP-43 was probed (1:1000) with mouse monoclonal (1D3) phospho-TDP-43 (Millipore). Mouse monoclonal (1:1000) CK1 (Abnova) and rabbit polyclonal CK2 (1:1000) antibodies (Abnova) were used to probe for casein kinases. Anti- α -Synuclein (1:1000) antibody (Chemicon International) was used to probe against α -Synuclein. Anti-Tau (1:1000) antibodies (Chemicon International) were used to probe for different Tau epitopes. Western blots were quantified by densitometry using Quantity One 4.6.3 software (Bio Rad).

Immunocytochemical and histological analysis of brain sections

Immunohistochemistry was performed on 20 micron-thick brain sections. TDP-43 was probed (1:200) with Rabbit polyclonal (ProteinTech) or (1:200) mouse monoclonal (Abnova) antibodies. Staining against human $A\beta_{1-42}$ was performed using (1:200) rabbit polyclonal antibody (Zymed). TDP-43 staining was coupled with nuclear DAPI staining to determine nuclear and cytosolic immunoreactivity of TDP-43.

Acknowledgments

This work was supported by NIH AG30378 award to Dr. Charbel E-H Moussa.

Abbreviations

PD	Parkinson's disease
AD	Alzheimer's disease
FTLD-U	Fronto-temporal lobar dementia with ubiquitin positive inclusions
ALS	Amyotrophic Lateral Sclerosis
MND	Motor Neuron Disease
TDP-43	Tar-DNA binding protein-43
Aβ	β -amyloid
CK1	Casein kinase 1

References

- Amador-Ortiz C, Lin WL, Ahmed Z, Personett D, Davies P, Duara R, Graff-Radford NR, Hutton ML, Dickson DW. TDP-43 immunoreactivity in hippocampal sclerosis and Alzheimer's disease. *Ann Neurol*. 2007; 61:435–45. [PubMed: 17469117]
- Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, Mann D, Tsuchiya K, Yoshida M, Hashizume Y, Oda T. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun*. 2006; 351:602–11. [PubMed: 17084815]
- Ardley HC, Tan NG, Rose SA, Markham AF, Robinson PA. Features of the parkin/ariadne-like ubiquitin ligase, HHARI, that regulate its interaction with the ubiquitin-conjugating enzyme, Ubch7. *J Biol Chem*. 2001; 276:19640–7. [PubMed: 11278816]
- Ayala YM, Pantano S, D'Ambrogio A, Buratti E, Brindisi A, Marchetti C, Romano M, Baralle FE. Human, *Drosophila*, and *C.elegans* TDP43: nucleic acid binding properties and splicing regulatory function. *J Mol Biol*. 2005; 348:575–88. [PubMed: 15826655]
- Bennett MC, Bishop JF, Leng Y, Chock PB, Chase TN, Mouradian MM. Degradation of alpha-synuclein by proteasome. *J Biol Chem*. 1999; 274:33855–8. [PubMed: 10567343]
- Buratti E, Brindisi A, Giombi M, Tisminetzky S, Ayala YM, Baralle FE. TDP-43 binds heterogeneous nuclear ribonucleoprotein A/B through its C-terminal tail: an important region for the inhibition of cystic fibrosis transmembrane conductance regulator exon 9 splicing. *J Biol Chem*. 2005; 280:37572–84. [PubMed: 16157593]
- Buratti E, Baralle FE. Multiple roles of TDP-43 in gene expression, splicing regulation, and human disease. *Front Biosci*. 2008; 13:867–78. [PubMed: 17981595]
- Burns MP, Zhang L, Rebeck GW, Querfurth HW, Moussa CE. Parkin promotes intracellular Abeta1-42 clearance. *Hum Mol Genet*. 2009; 18:3206–16. [PubMed: 19483198]
- Caccamo A, Majumder S, Deng JJ, Bai Y, Thornton FB, Oddo S. Rapamycin rescues TDP-43 mislocalization and the associated low molecular mass neurofilament instability. *J Biol Chem*. 2009; 284:27416–24. [PubMed: 19651785]
- Caccamo A, Magri A, Oddo S. Age-dependent changes in TDP-43 levels in a mouse model of Alzheimer disease are linked to Abeta oligomers accumulation. *Mol Neurodegener*. 2010; 5:51. [PubMed: 21070634]
- Cook DG, Forman MS, Sung JC, Leight S, Kolson DL, Iwatsubo T, Lee VM, Doms RW. Alzheimer's A beta(1-42) is generated in the endoplasmic reticulum/intermediate compartment of NT2N cells. *Nat Med*. 1997; 3:1021–3. [PubMed: 9288730]

- Davidson Y, Kelley T, Mackenzie IR, Pickering-Brown S, Du Plessis D, Neary D, Snowden JS, Mann DM. Ubiquitinated pathological lesions in frontotemporal lobar degeneration contain the TAR DNA-binding protein, TDP-43. *Acta Neuropathol.* 2007; 113:521–33. [PubMed: 17219193]
- Forman MS, Farmer J, Johnson JK, Clark CM, Arnold SE, Coslett HB, Chatterjee A, Hurtig HI, Karlawish JH, Rosen HJ, Van Deerlin V, Lee VM, Miller BL, Trojanowski JQ, Grossman M. Frontotemporal dementia: clinicopathological correlations. *Ann Neurol.* 2006; 59:952–62. [PubMed: 16718704]
- Geser F, Winton MJ, Kwong LK, Xu Y, Xie SX, Igaz LM, Garruto RM, Perl DP, Galasko D, Lee VM, Trojanowski JQ. Pathological TDP-43 in parkinsonism-dementia complex and amyotrophic lateral sclerosis of Guam. *Acta Neuropathol.* 2008; 115:133–45. [PubMed: 17713769]
- Gitcho MA, Baloh RH, Chakraborty S, Mayo K, Norton JB, Levitch D, Hatanpaa KJ, White CL 3rd, Bigio EH, Caselli R, Baker M, Al-Lozi MT, Morris JC, Pestronk A, Rademakers R, Goate AM, Cairns NJ. TDP-43 A315T mutation in familial motor neuron disease. *Ann Neurol.* 2008; 63:535–8. [PubMed: 18288693]
- Gouras GK, Tsai J, Naslund J, Vincent B, Edgar M, Checler F, Greenfield JP, Haroutunian V, Buxbaum JD, Xu H, Greengard P, Relkin NR. Intraneuronal A β 42 accumulation in human brain. *Am J Pathol.* 2000; 156:15–20. [PubMed: 10623648]
- Greenfield JP, Tsai J, Gouras GK, Hai B, Thinakaran G, Checler F, Sisodia SS, Greengard P, Xu H. Endoplasmic reticulum and trans-Golgi network generate distinct populations of Alzheimer beta-amyloid peptides. *Proc Natl Acad Sci U S A.* 1999; 96:742–7. [PubMed: 9892704]
- Gros-Louis F, Gaspar C, Rouleau GA. Genetics of familial and sporadic amyotrophic lateral sclerosis. *Biochim Biophys Acta.* 2006; 1762:956–72. [PubMed: 16503123]
- Haass C, Schlossmacher MG, Hung AY, Vigo-Pelfrey C, Mellon A, Ostaszewski BL, Lieberburg I, Koo EH, Schenk D, Teplow DB, et al. Amyloid beta-peptide is produced by cultured cells during normal metabolism. *Nature.* 1992; 359:322–5. [PubMed: 1383826]
- Haninara T, Amano N, Takahashi T, Nagatomo H, Yagashita S. Distribution of tangles and threads in the cerebral cortex in progressive supranuclear palsy. *Neuropathol Appl Neurobiol.* 1995; 21:319–26. [PubMed: 7494600]
- Hardy J, Selkoe DJ. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science.* 2002; 297:353–6. [PubMed: 12130773]
- Hasegawa M, Arai T, Akiyama H, Nonaka T, Mori H, Hashimoto T, Yamazaki M, Oyanagi K. TDP-43 is deposited in the Guam parkinsonism-dementia complex brains. *Brain.* 2007; 130:1386–94. [PubMed: 17439983]
- Hasegawa M, Arai T, Nonaka T, Kametani F, Yoshida M, Hashizume Y, Beach TG, Buratti E, Baralle F, Morita M, Nakano I, Oda T, Tsuchiya K, Akiyama H. Phosphorylated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Ann Neurol.* 2008; 64:60–70. [PubMed: 18546284]
- Hof PR, Delacourte A, Bouras C. Distribution of cortical neurofibrillary tangles in progressive supranuclear palsy: a quantitative analysis of six cases. *Acta Neuropathol.* 1992; 84:45–51. [PubMed: 1502881]
- Hyun DH, Lee M, Hattori N, Kubo S, Mizuno Y, Halliwell B, Jenner P. Effect of wild-type or mutant Parkin on oxidative damage, nitric oxide, antioxidant defenses, and the proteasome. *J Biol Chem.* 2002; 277:28572–7. [PubMed: 12034719]
- Ito K, Arai K, Yoshiyama Y, Kashiwado K, Sakakibara Y, Hattori T. Astrocytic tau pathology positively correlates with neurofibrillary tangle density in progressive supranuclear palsy. *Acta Neuropathol.* 2008; 115:623–8. [PubMed: 18427815]
- Khandelwal PJ, Dumanis SB, Feng LR, Maguire-Zeiss K, Rebeck G, Lashuel HA, Moussa CE. Parkinson-related parkin reduces alpha-Synuclein phosphorylation in a gene transfer model. *Mol Neurodegener.* 2010; 5:47. [PubMed: 21050448]
- Koo EH, Squazzo SL. Evidence that production and release of amyloid beta-protein involves the endocytic pathway. *J Biol Chem.* 1994; 269:17386–9. [PubMed: 8021238]
- Lee SJ, Liyanage U, Bickel PE, Xia W, Lansbury PT Jr, Kosik KS. A detergent-insoluble membrane compartment contains A β in vivo. *Nat Med.* 1998; 4:730–4. [PubMed: 9623986]

- Lee VM, Goedert M, Trojanowski JQ. Neurodegenerative tauopathies. *Annu Rev Neurosci.* 2001; 24:1121–59. [PubMed: 11520930]
- Lomen-Hoerth C, Anderson T, Miller B. The overlap of amyotrophic lateral sclerosis and frontotemporal dementia. *Neurology.* 2002; 59:1077–9. [PubMed: 12370467]
- Lomen-Hoerth C, Murphy J, Langmore S, Kramer JH, Olney RK, Miller B. Are amyotrophic lateral sclerosis patients cognitively normal? *Neurology.* 2003; 60:1094–7. [PubMed: 12682312]
- Mackenzie IR, Bigio EH, Ince PG, Geser F, Neumann M, Cairns NJ, Kwong LK, Forman MS, Ravits J, Stewart H, Eisen A, McClusky L, Kretschmar HA, Monoranu CM, Highley JR, Kirby J, Siddique T, Shaw PJ, Lee VM, Trojanowski JQ. Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. *Ann Neurol.* 2007; 61:427–34. [PubMed: 17469116]
- Morales B, Martinez A, Gonzalo I, Vidal L, Ros R, Gomez-Tortosa E, Rabano A, Ampuero I, Sanchez M, Hoenicka J, Garcia De Yebenes J. Steele-Richardson-Olszewski syndrome in a patient with a single C212Y mutation in the parkin protein. *Mov Disord.* 2002; 17:1374–80. [PubMed: 12465088]
- Morett E, Bork P. A novel transactivation domain in parkin. *Trends Biochem Sci.* 1999; 24:229–31. [PubMed: 10366851]
- Morita M, Al-Chalabi A, Andersen PM, Hosler B, Sapp P, Englund E, Mitchell JE, Habgood JJ, de Belleruche J, Xi J, Jongjaroenprasert W, Horvitz HR, Gunnarsson LG, Brown RH Jr. A locus on chromosome 9p confers susceptibility to ALS and frontotemporal dementia. *Neurology.* 2006; 66:839–44. [PubMed: 16421333]
- Murray B, Lynch T, Farrell M. Clinicopathological features of the tauopathies. *Biochem Soc Trans.* 2005; 33:595–9. [PubMed: 16042552]
- Nakashima-Yasuda H, Uryu K, Robinson J, Xie SX, Hurtig H, Duda JE, Arnold SE, Siderowf A, Grossman M, Leverenz JB, Woltjer R, Lopez OL, Hamilton R, Tsuang DW, Galasko D, Masliah E, Kaye J, Clark CM, Montine TJ, Lee VM, Trojanowski JQ. Co-morbidity of TDP-43 proteinopathy in Lewy body related diseases. *Acta Neuropathol.* 2007; 114:221–9. [PubMed: 17653732]
- Neary D, Snowden JS, Mann DM, Northen B, Goulding PJ, Macdermott N. Frontal lobe dementia and motor neuron disease. *J Neurol Neurosurg Psychiatry.* 1990; 53:23–32. [PubMed: 2303828]
- Neary D, Snowden JS, Mann DM. Classification and description of frontotemporal dementias. *Ann N Y Acad Sci.* 2000; 920:46–51. [PubMed: 11193176]
- Neumann M, Sampathu DM, Kwong LK, Truax AC, Micsenyi MC, Chou TT, Bruce J, Schuck T, Grossman M, Clark CM, McCluskey LF, Miller BL, Masliah E, Mackenzie IR, Feldman H, Feiden W, Kretschmar HA, Trojanowski JQ, Lee VM. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science.* 2006; 314:130–3. [PubMed: 17023659]
- Neumann M, Kwong LK, Sampathu DM, Trojanowski JQ, Lee VM. TDP-43 proteinopathy in frontotemporal lobar degeneration and amyotrophic lateral sclerosis: protein misfolding diseases without amyloidosis. *Arch Neurol.* 2007a; 64:1388–94. [PubMed: 17923623]
- Neumann M, Mackenzie IR, Cairns NJ, Boyer PJ, Markesbery WR, Smith CD, Taylor JP, Kretschmar HA, Kimonis VE, Forman MS. TDP-43 in the ubiquitin pathology of frontotemporal dementia with VCP gene mutations. *J Neuropathol Exp Neurol.* 2007b; 66:152–7. [PubMed: 17279000]
- Pasinelli P, Brown RH. Molecular biology of amyotrophic lateral sclerosis: insights from genetics. *Nat Rev Neurosci.* 2006; 7:710–23. [PubMed: 16924260]
- Ratnavalli E, Brayne C, Dawson K, Hodges JR. The prevalence of frontotemporal dementia. *Neurology.* 2002; 58:1615–21. [PubMed: 12058088]
- Rebeck GW, Hoe HS, Moussa CE. [beta]-Amyloid1-42 gene transfer model exhibits intraneuronal amyloid, gliosis, tau phosphorylation, and neuronal loss. *J Biol Chem.* 2010
- Ros R, Ampuero I, Garcia de Yebenes J. Parkin polymorphisms in progressive supranuclear palsy. *J Neurol Sci.* 2008; 268:176–8. [PubMed: 18022644]
- Rosen DR. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature.* 1993; 364:362. [PubMed: 8332197]

- Sanchez MP, Gonzalo I, Avila J, De Yebenes JG. Progressive supranuclear palsy and tau hyperphosphorylation in a patient with a C212Y parkin mutation. *J Alzheimers Dis.* 2002; 4:399–404. [PubMed: 12446971]
- Skovronsky DM, Doms RW, Lee VM. Detection of a novel intraneuronal pool of insoluble amyloid beta protein that accumulates with time in culture. *J Cell Biol.* 1998; 141:1031–9. [PubMed: 9585420]
- Snowden JS, Neary D, Mann DM. Frontotemporal dementia. *Br J Psychiatry.* 2002; 180:140–3. [PubMed: 11823324]
- Sreedharan J, Blair IP, Tripathi VB, Hu X, Vance C, Rogelj B, Ackerley S, Durnall JC, Williams KL, Buratti E, Baralle F, de Belleruche J, Mitchell JD, Leigh PN, Al-Chalabi A, Miller CC, Nicholson G, Shaw CE. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. *Science.* 2008; 319:1668–72. [PubMed: 18309045]
- Talbot K, Ansorge O. Recent advances in the genetics of amyotrophic lateral sclerosis and frontotemporal dementia: common pathways in neurodegenerative disease. *Hum Mol Genet.* 2006; 15:R182–7. Spec No 2. [PubMed: 16987882]
- Tan CF, Eguchi H, Tagawa A, Onodera O, Iwasaki T, Tsujino A, Nishizawa M, Kakita A, Takahashi H. TDP-43 immunoreactivity in neuronal inclusions in familial amyotrophic lateral sclerosis with or without SOD1 gene mutation. *Acta Neuropathol.* 2007; 113:535–42. [PubMed: 17333220]
- Tofaris GK, Layfield R, Spillantini MG. alpha-synuclein metabolism and aggregation is linked to ubiquitin-independent degradation by the proteasome. *FEBS Lett.* 2001; 509:22–6. [PubMed: 11734199]
- van de Warrenburg BP, Lammens M, Lucking CB, Deneffe P, Wesseling P, Booij J, Praamstra P, Quinn N, Brice A, Horstink MW. Clinical and pathologic abnormalities in a family with parkinsonism and parkin gene mutations. *Neurology.* 2001; 56:555–7. [PubMed: 11222808]
- Wang HY, Wang IF, Bose J, Shen CK. Structural diversity and functional implications of the eukaryotic TDP gene family. *Genomics.* 2004; 83:130–9. [PubMed: 14667816]
- Wegorzewska I, Bell S, Cairns NJ, Miller TM, Baloh RH. TDP-43 mutant transgenic mice develop features of ALS and frontotemporal lobar degeneration. *Proc Natl Acad Sci U S A.* 2009; 106:18809–14. [PubMed: 19833869]
- Wilson CA, Doms RW, Lee VM. Intracellular APP processing and A beta production in Alzheimer disease. *J Neuropathol Exp Neurol.* 1999; 58:787–94. [PubMed: 10446803]
- Winton MJ, Igaz LM, Wong MM, Kwong LK, Trojanowski JQ, Lee VM. Disturbance of nuclear and cytoplasmic TAR DNA-binding protein (TDP-43) induces disease-like redistribution, sequestration, and aggregate formation. *J Biol Chem.* 2008; 283:13302–9. [PubMed: 18305110]
- Xu H, Sweeney D, Wang R, Thinakaran G, Lo AC, Sisodia SS, Greengard P, Gandy S. Generation of Alzheimer beta-amyloid protein in the trans-Golgi network in the apparent absence of vesicle formation. *Proc Natl Acad Sci U S A.* 1997; 94:3748–52. [PubMed: 9108049]
- Zhang YJ, Xu YF, Dickey CA, Buratti E, Baralle F, Bailey R, Pickering-Brown S, Dickson D, Petrucelli L. Progranulin mediates caspase-dependent cleavage of TAR DNA binding protein-43. *J Neurosci.* 2007; 27:10530–4. [PubMed: 17898224]
- Zhang YJ, Xu YF, Cook C, Gendron TF, Roettges P, Link CD, Lin WL, Tong J, Castanedes-Casey M, Ash P, Gass J, Rangachari V, Buratti E, Baralle F, Golde TE, Dickson DW, Petrucelli L. Aberrant cleavage of TDP-43 enhances aggregation and cellular toxicity. *Proc Natl Acad Sci U S A.* 2009; 106:7607–12. [PubMed: 19383787]

Fig. 1. TPD-43 pathology in induced in brain autopsies from AD patients

A). WB of lysates from human motor cortex were probed with anti-APP (C1.61) antibody (upper blot). Anti-TDP-43 (Abnova) antibody (upper middle blot) showed an increase in TDP-43 level, the appearance of a small ~15kDa band and a protein smear on the upper gel in AD brains not control. Anti phospho-TDP-43 (pTDP-43) antibody (lower middle blot) shows an increase in pTDP-43 levels and cleavage and aggregation in AD not control. Bottom blot is an actin loading control, molecular weight markers are indicated. **B).** Densitometric analysis of blots, Asterisk is significantly different to control, $P < 0.05$, Neuman Keuls with multiple comparison, $N = 7$ control; 12 AD patients.

Fig. 2. Intracellular A β ₁₋₄₂ induces TDP-43 protein up-regulation, cleavage, and aggregation in gene transfer animal models

A). WB analysis of brain lysates on 4-12% NuPAGE gel probed with anti-parkin antibody (PRK8) and anti-TDP-43 antibody (Abnova) showing an increase in the level of TDP-43 expression, cleavage and aggregation in lentiviral-A β ₁₋₄₂ compared to lentiviral LacZ, parkin or α -Synuclein injected brains. Middle blot shows A β ₁₋₄₂ and bottom blot shows α -Synuclein expression. **B).** WB analysis with anti p-TDP-43 (Millipore) antibody (upper blot) shows an increase in the level of pTDP-43 expression (asterisks), and cleavage of TDP-43 in lentiviral-A β ₁₋₄₂ compared to lentiviral LacZ or α -Synuclein injected brains. Middle blots show casein kinase 1 and 2 (CK1 and CK2) levels, and bottom blot shows actin loading control. **C).** Blots show parkin expression and TDP-43 immunoreactivity in brain lysates of 3xTg-AD mice. **D).** Blots show TDP-43 immunoreactivity in brain lysates of 3xTg-AD mice compared to non-transgenic wild type controls. **E).** Shows blots of GSK-3 β and different Tau epitopes in 3xTg-AD mice. Lentiviral- α -Synuclein, Lv-Syn, Lentiviral LacZ: Lv-LacZ, Lentiviral A β ₁₋₄₂: Lv-A β ₁₋₄₂, N=4 animals per treatment.

Fig. 3. Intracellular A β_{1-42} increases cytosolic accumulation of TDP-43

A). Staining of 20 micron thick cortical rat brain sections injected with 2×10^{10} m.o.i LacZ lentivirus and **B).** 1×10^{10} each of lentiviral LacZ and lentiviral A β_{1-42} . **C)** High magnification (60 $\times$) of 20 micron-thick sections of rat motor cortex stained with DAPI and TPD-43 antibody (ProteinTech) in lentiviral LacZ and **D).** A β_{1-42} injected rat brains. **E).** Co-staining against human A β_{1-42} (Zymed) and **F).** TDP-43 (Abnova) shows **G).** co-localization of these proteins in A β_{1-42} expressing cells and up-regulation and cytosolic accumulation of TDP-43. Lentiviral lacZ: Lv-LacZ, Lentiviral A β_{1-42} : Lv-A β_{1-42} . N=4 animals per treatment.