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Review Article

The Interface between Cytoskeletal Aberrations and Mitochondrial Dysfunction in Alzheimer's Disease and Related Disorders

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The major defining pathological hallmarks of Alzheimer's disease (AD) are the accumulations of Aβ in senile plaques and hyperphosphorylated tau in neurofibrillary tangles and neuropil threads. Recent studies indicate that rather than these insoluble lesions, the soluble Aβ oligomers and hyperphosphorylated tau are the toxic agents of AD pathology. Such pathological protein species are accompanied by cytoskeletal changes, mitochondrial dysfunction, Ca^{2+} dysregulation, and oxidative stress. In this review, we discuss how the binding of Aβ to various integrins, defects in downstream focal adhesion signaling, and activation of cofilin can impact mitochondrial dysfunction, cytoskeletal changes, and tau pathology induced by Aβ oligomers. Such pathological consequences can also feedback to further activate cofilin to promote cofilin pathology. We also suggest that the mechanism of Aβ generation by the endocytosis of APP is mechanistically linked with perturbations in integrin-based focal adhesion signaling, as APP, LRP, and β-integrins are physically associated with each other.

Key words: integrin, focal adhesion, cofilin, amyloid, mitochondria, cytoskeleton

ALZHEIMER'S DISEASE DEFINITION AND PREVALENCE

Alzheimer's disease (AD) is the most common age-related neurodegenerative condition, accounting for up to 70% of all cases of dementia. Prevalence rates of AD range from ~10% of individuals >65 and up to 50% of individuals greater than 85 years of age. Early stages of AD are presented by short-term memory deficits, which steadily decline with worsening dementia clinically manifested by long-term memory loss, language disturbances,

changes in personality, attention deficits, hallucinations, and reasoning impairments. The clinical course ranges usually from 5 to 15 years.

PATHOLOGICAL HALLMARKS OF ALZHEIMER'S DISEASE

The major defining pathological hallmarks of Alzheimer's disease (AD) are the accumulations of amyloid β (Aβ) and hyperphosphorylated tau in senile plaques and neurofibrillary tangles, respectively. Aβ is a neurotoxic peptide derived from β- and γ-secretase cleavages of the amyloid precursor protein (APP). The vast majority of APP is constitutively cleaved in the middle of the Aβ sequence by α-secretase (ADAM10/TACE/ADAM17) in the non-amyloidogenic pathway, thereby abrogating

the generation of an intact Aβ peptide. Alternatively, a small proportion of APP is cleaved in the amyloidogenic pathway, leading to the secretion of Aβ peptides (37 to 42 amino acids) via two proteolytic enzymes, β- and γ-secretase, known as BACE1 and presenilin, respectively (De Strooper and Annaert, 2000). The proteolytic processing of APP to generate Aβ requires endocytosis from the cell surface and localization to cholesterol-rich membrane rafts where both BACE1 and the presenilin complex are enriched (Koo and Squazzo, 1994; Perez et al., 1999; Wahrle et al., 2002).

Neurofibrillary tangles and neuropil threads are intracellular inclusions principally composed of the microtubule associated protein tau in hyperphosphorylated form. Hyperphosphorylated tau assembles into twisted filaments ultrastructurally seen as paired helical filaments or PHFs, which are immunopositive with AD-specific diagnostic antibodies, such as AT100 or AT8 (Avila et al., 2004). Tau proteins normally function to stabilize microtubules and are usually seen in somatoaxonal compartments. However during early stages of AD pathogenesis, tau becomes hyperphosphorylated, which weakens its association with microtubules, and is frequently detected in somatodendritic compartments. Thus, hyperphosphorylated tau is unable to stabilize microtubules, mislocalizes to dendritic compartments, and becomes more prone to self-assembly into PHFs (Gendron and Petrucelli, 2009).

GENETICS OF ALZHEIMER' DISEASE AND THE AMYLOID HYPOTHESIS

Twenty years ago in 1991, the amyloid hypothesis was postulated that the Aβ deposits are fundamental causes of AD rather than simply pathological hallmarks of the disease (Hardy and Allsop, 1991). This hypothesis was initially supported by the identification of mutations in APP that co-segregated with early onset familial AD (FAD). Importantly, all of the FAD mutations identified thus far are concentrated near the β-secretase or γ-secretase cleavage sites in APP such that either total Aβ or the more pathogenic Aβ42 peptide is elevated. While γ-secretase cleavage of APP at Aβ residue 42 (Aβ42) is a minor species (~10%) compared to Aβ40 (>80%), numerous studies have shown that Aβ42 is more neurotoxic and is required to efficiently seed the aggregation of Aβ40 (Glabe, 2008). Furthermore, individuals with trisomy 21 (Down's syndrome) with an extra copy of the APP gene on chromosome 21 invariably exhibit AD pathology by the 4th decade of life. Closer examination of Down's syndrome brains showed that Aβ42 containing senile plaques develop much earlier than neurofibrillary tangles, further supporting the amyloid hypothesis

(Hyman, 1992; Hyman et al., 1995). In 1995, mutations in two homologous genes, Presenilin 1 (PS1) and Presenilin 2 (PS2) were discovered that co-segregated with the most aggressive forms of autosomal dominant early-onset FAD. Thus far, more than 100 different independent FAD mutations in PS1 and PS2 have been identified (St George-Hyslop and Petit, 2005). Further adding support to the amyloid hypothesis, all of the presenilin mutations studied were found to increase the more pathogenic and faster aggregating Aβ42 peptide. Although mutations in *APP, PS1,* and *PS2* account for the majority of early-onset familial AD, the vast majority (>95%) of AD is the late-onset sporadic form that cannot be explained by a single gene defect. Nevertheless, genetic risk factors play an important modulatory role in the onset and severity of AD pathogenesis. The best known and strongest genetic risk factor identified to date is the ε4 allele of the gene coding for Apolipoprotein E (APOE). The APOE ε4 allele increases the risk for AD by ~3-fold among hemizygotes and by ~15-fold among homozygotes (Holtzman, 2001). Further supporting the amyloid hypothesis, apoE4 promotes the aggregation of Aβ far more efficiently than apoE3, the most common nonpathogenic variant (Strittmatter et al., 1993). Therefore, all known and confirmed genetic component of AD either increase total Aβ, the more pathogenic Aβ42 peptide, or aggregation states of Aβ.

SOLUBLE Aβ OLIGOMER HYPOTHESIS AND TAU PATHOLOGY

Recent biochemical and cellular studies on Aβ-induced neuro-toxicity have uncovered that distinct states or species of Aβ have different biological properties. First, Aβ42, while at much lower concentration than Aβ40, aggregates faster and seeds the aggregation of Aβ40 (Glabe, 2008). Transgenic mice engineered to produce only Aβ40 or Aβ42 cleaved from the familial British and Danish Dementia-related BRI protein demonstrates that production of Aβ40 cannot form aggregates into plaques even by 18 months of age, while a lesser concentration of Aβ42 induces robust plaque formation even at 12 months of age. Furthermore, the BRI-Aβ42 mice crossed with APP Tg2576 mice bearing the "Swedish" mutation exponentially exacerbates amyloid plaque for-mation (McGowan et al., 2005). Second, Aβ can exist as soluble monomers, dimers, trimers, and higher order oligomers (also known as Aβ-derived diffusible ligands; ADDLs) prior to forming protofibrils and insoluble amyloid fibrils. These pathological so-luble Aβ oligomers cause synaptic dysfunction to neurons even at picomolar concentrations. Studies have shown that SDS-stable dimers and trimers derived from the 7PA2 cell line expressing an FAD APP mutation impair long term potentiation (LTP), the physiological correlate of learning and memory, at subna-

nomolar concentrations (Walsh et al., 2002). Furthermore, soluble SDS-resistant Aβ dimers derived from AD brains lead to hyperphosphorylation of tau and neuritic degeneration in primary hippocampal neurons at picomolar concentrations (Jin et al., 2011). Recently, a new mutation in APP was identified in a Japanese family with AD-type dementia. This mutation occurred as a deletion of residue 22 (glutamic acid) of the Aβ peptide (E22Δ). Biochemically, the E22Δ mutation is more resistant to degradation, unable to form fibrils, but far more prone to self-association as Aβ oligomers in the form of SDS-stable dimers and trimers (Tomiyama et al., 2008). Expression of this APP mutation in transgenic mice leads to learning and memory deficits associated with impaired LTP, enhanced neuroinflammation, and tau hyperphosphorylation in the absence of any thioflavin S positive amyloid plaques. However, accumulation of intracellular E22Δ Aβ oligomers are detected in an age-dependent fashion, indicating that Aβ oligomers are sufficient and fibrillar amyloid deposition is not necessary for Aβ-induced neurotoxicity and learning and memory deficits (Nishitsuji et al., 2009). Indeed, a recent study showed that highly compacted amyloid plaques may actually be protective. Loss of one copy of IGF-1 receptor, which lengthens lifespan, was shown to be protective by promoting faster aggregation of Aβ and increasing the density of amyloid plaques, thereby sequestering toxic soluble Aβ oligomer species into amyloid fibrils (Cohen et al., 2009). While it appears that amyloid fibrils per se might be less neurotoxic than soluble Aβ oligomers, it is important to note that amyloid fibrils can disaggregate into soluble Aβ oligomers, and thus, may serve as stable sources of these neurotoxic species.

Numerous studies have shown that Aβ promotes the hyperphosphorylation of tau *in vitro* and *in vivo* (Avila et al., 2004; Gendron and Petrucelli, 2009). Furthermore, Aβ also increases the number of neurofibrillary tangles in transgenic mice engineered to express a Frontotemporal Dementia (FTDP-17) tau mutation (Lewis et al., 2001). Moreover, depletion of Aβ by injection of an antibody directed against Aβ also leads to reduced tau pathology in the APP/tau/presenilin-1 mutant triple transgenic mice (Oddo et al., 2004), further supporting the model that Aβ promotes tau pathology *in vivo*. In addition, recent studies have demonstrated that many of the toxic effects of Aβ require tau expression, indicating that tau is indeed downstream of Aβ-induced neurotoxic signals. Neurite retraction and progressive neuronal atrophy are seen when neurons are treated with Aβ but not in neurons derived from tau knockout mice (Rapoport et al., 2002; Jin et al., 2011). Furthermore, learning and memory impairment as well as high sensitivity to excitotoxin treatment are present in mutant APP transgenic mice but not in the same transgenic mice on a tau homozygous knockout background,

even though the level of Aβ deposition is unaffected by tau (Roberson et al., 2007). Importantly, Aβ induces impairments in LTP and axonal transport of mitochondria; however, such impairments are not seen in tau knockout neurons (Shipton et al., 2011; Vossel et al., 2011), indicating that tau is required for multiple facets of Aβ-induced neurotoxicity. These results all indicate that Aβ-induced toxic signals are transmitted via tau. But what might be a mechanism of tau in Aβ/ tau-mediated neurotoxicity? A recent study demonstrated that Aβ oligomers rapidly lead to disassembly of microtubules but only in cells expressing tau (King et al., 2006). This is particularly important in brain, because tau is highly expressed in neurons. Furthermore, the microtubule network is critical for such processes as axonal transport and ultimately synaptic transmission. One of the earliest changes in tau pathology seen in AD is the mislocalization of tau from somatoaxonal to somatodendritic compartments (Avila et al., 2004; Gendron and Petrucelli, 2009). An important study recently demonstrated that hyperphosphorylation of tau is linked to reduced affinity for microtubules and mislocalization into dendritic spines where tau induces removal of various AMPA and NMDA receptors critical for excitatory synaptic transmission. Loss of tau phosphorylation fails to mislocalize tau into dendritic spines and affect excitatory synaptic transmission (Hoover et al., 2010). Therefore, Aβ-induced hyperphosphorylation of tau appears to explain both microtubule disassembly and impaired excitatory synaptic transmission. It is important to note that, like soluble Aβ oligomers, soluble hyperphosphorylated tau rather than PHF tau may be the toxic species, since turning off FTD mutant tau expression in an inducible transgenic model does not remove insoluble PHF-1 positive tangle-like structures over several months but improves learning and memory (Santacruz et al., 2005).

MITOCHONDRIAL DYSFUNCTION IN AD: Aβ, CA²⁺ DYSREGULATION AND ROS ACCUMULATION

As the human brain utilizes 20% of total body oxygen consumption, mitochondria, the cellular energy plant, is of great importance when it comes the mechanisms of neurodegeneration. Mitochondria serve as important reservoirs of Ca^{2+} and apoptotic proteins and produce reactive oxygen species (ROS), all of which have important implications in AD pathogenesis. The role of Ca^{2+} signaling is essential for neuronal survival and function in that they use Ca^{2+} in generating membrane excitability, releasing neurotransmitters, activity-dependent gene expression, neuronal growth and differentiation, and triggering apoptosis (Bezprozvanny and Mattson, 2008). Intracellular Ca^{2+} homeostasis is tightly maintained by the interplay among various Ca^{2+} channels and pro-

teins within different cellular compartments, including the Ca^{2+} uniporter (MCU) in mitochondria (Bezprozvanny, 2009). As Ca^{2+} homeostasis is regulated through multiple mechanisms, the implications of its regulation to the development of AD are also varied.

Mitochondria play essential roles in regulating Ca^{2+} homeostasis and ROS generation, both of which are pathologically linked to neurotoxicity. When Ca^{2+} levels increase in the cytosol, mitochondria potently take up Ca^{2+} through the mitochondrial Ca^{2+} uniporter (MCU), resulting in Ca^{2+} buffering and increased metabolism and production of ATP (Bezprozvanny and Mattson, 2008). However if excess amounts of Ca^{2+} enter the mitochondria (calcium overload), it leads to decreased mitochondrial membrane potential (MMP) and oxidative stress. This, in turn, results in formation of the mitochondrial permeability-transition pore (mtPTP) and failure to exert Ca^{2+} buffering capacity, leading to secondary excitotoxicity (Hengartner, 2000). ROS are also produced as a result of the opening of the mtPTP, as antioxidants such a glutathione can exit the mitochondria, resulting in the failure to neutralize ROS. Moreover, loss of components of the electron transport chain (ETC) such as cytochrome c and others may occur through the mtPTP, resulting in activation of caspases 3, 6, 7, 8 and 9 and increased ROS production (Li et al., 1997; Bossy-Wetzel et al., 1998; Granville et al., 1998). ROS, at low to moderate concentrations, is an important mediator of defense mechanisms against infectious agents, various cellular signaling events, and synaptic function. However, if the ROS production exceeds the amount that can be neutralized by anti-oxidant mechanisms, it eventually results in mitochondrial dysfunction and neuronal damage (Andreyev et al., 2005), the molecular targets of which are DNA, lipids, and protein machineries within the cell and mitochondria (Knight, 1997). Interestingly, increasing mitochondrial oxidative stress induced by deficiency of mitochondrial superoxide dismutase 2 leads to high levels of Ser-396 phosphorylated tau (Melov et al., 2007), indicating that mitochondrial oxidative damage also impacts tau pathology.

The role of mitochondrial ROS as inducers of Ca^{2+} dysregulation is well-established. On the other hand, a major cause of ROS production is also Ca^{2+} dysregulation. Thus, oxidative stress and Ca^{2+} regulation are intricately linked and can cooperatively contribute to AD pathogenesis. It has been shown that Aβ oligomers can form Ca^{2+}-permeable channels in the plasma membranes (Arispe et al., 1993) and that the association of Aβ to membranes is enhanced in the presence of excess phosphatidylserine (PtdS) (Lee et al., 2002a). Multiple studies have demonstrated that Aβ can dysregulate Ca^{2+} homeostasis via several Ca^{2+} signaling components (Bezprozvanny, 2009): calcineurin

(Kuchibhotla et al., 2008), NMDARs (De Felice et al., 2007), AMPARs (Hsieh et al., 2006), and P/Q-type VGCCs (Nimmrich et al., 2008). Furthermore, FAD mutations in presenilins (PS), which potentiate Aβ42 generation, induce excessive Ca^{2+} release from the ER through IP3 and ryanodine receptors (Cheung et al., 2008; Rybalchenko et al., 2008). In AD brains, intracellular Ca^{2+} levels are positively correlated with neurofibrillary tangle formation (McKee et al., 1990; Nixon, 2003). As such, it is clear that dysregulation of Ca^{2+} dynamics is an important component of AD pathogenesis. It has been shown that Aβ decreases mitochondrial membrane potential and increases respiration uncoupling and mitochondrial ROS (Moreira et al., 2001) along with the inhibition of complexes I, III, and IV of the mitochondrial respiratory chain (Pereira et al., 1998). Other studies have demonstrated that Aβ treatment promotes caspase 3, 8, and 9 activation (Fossati et al., 2010; Wang et al., 2010). Furthermore, Aβ induces abnormal mitochondrial dynamics. Specifically, Aβ (25-35) peptide impairs axonal transport of mitochondria, attenuates mitochondrial motility, and alters mitochondrial distribution in mouse hippocampal neurons, resulting in synaptic degeneration (Calkins and Reddy, 2011). A recent study demonstrated early deficits in synaptic mitochondria in FAD mutant APP transgenic mice: 1) synaptic mitochondrial dysfunction, 2) Aβ accumulation within mitochondria prior to extracellular Aβ deposition, and 3) impaired axonal transport of mitochondria (Du et al., 2010). Synapses are relatively more susceptible to Aβ-induced neurotoxicity, which is not surprising, because Aβ is mainly released by and accumulates in synapses (Mattson, 2004). Aβ induces Ca^{2+} dysregulation by membrane-associated oxidative stress and activates calcineurin, an important mediator of synaptic plasticity (Celsi et al., 2007). In addition, Aβ induces synaptic dystrophy and dysregulation at least in part due to decreased expression of NMDA and EphB2 receptors at the synapse, an event that can be corrected by NMDAR agonists (Lacor et al., 2007). Though the exact mechanisms of ROS-induced synaptic degeneration remain to be further elucidated, ROS is well known to play important roles in synaptic plasticity. Indeed, oxidation of cysteine residues on RynR increases Ca^{2+} release from the ER (Hidalgo, 2005) and oxidation of the same residues on NMDAR decreases the receptor activity (Lipton et al., 2002). Therefore, it appears that Aβ induces synaptic deficits via both increasing oxidative damage and dysregulating Ca^{2+} dynamics at the synapse and mitochondria, both of which cooperatively contribute to neurodegeneration in AD.

Tau pathology is also linked to mitochondrial dysfunction. Transgenic mice over-expressing the P301L mutant demonstrate a significant reduction in complex V and NADH-ubiquinone oxido-reductase activity together with impairments in mitochondrial

respiration and ATP synthesis (David et al., 2005). Tau can be cleaved by caspases 3, 7, and 8 after Asp-421, yielding a 421 residue tau fragment that assembles into PHF-like fibrils more rapidly than full length tau. Overexpression of this cleaved tau leads to a significant decrease in mitochondrial membrane potential and loss of mitochondrial membrane integrity (Quintanilla et al., 2009). Moreover, introduction of tau in mature hippocampal neurons results in degeneration of synapses by perturbing mitochondria transport and ATP levels at the synapse (Thies and Mandelkow, 2007). N-terminal tau fragments have toxic effects on mitochondria and lead to the mitochondrial dysfunction associated with impairments in oxidative phosphorylation by distorting the enzyme structure of complex V and the level of adenine nucleotide translocator, which ultimately perturbs ATP synthesis in mitochondria (Atlante et al., 2008). Interestingly, these N-terminal tau fragments are localized in AD synaptic mitochondria (Amadoro et al., 2010). Taken together, both Aβ and tau exert deleterious effects on mitochondria, events that involve ROS generation and Ca^{2+} dysregulation, which negatively impact synaptic function and neuronal viability.

Aβ NEUROTOXIC SIGNALING VIA INTEGRINS AND FOCAL ADHESIONS

Aβ oligomers are clearly neurotoxic at least in part by transmitting neurotoxic signals to tau. Furthermore, both Aβ and tau exert deleterious effects on mitochondria, resulting in ROS accumulation and Ca^{2+} dysregulation, which ultimately compromises synaptic function and neuronal viability. But what types of neuronal surface receptors and intracellular signals are needed to transmit these Aβ-induced neurotoxic messages to tau and mitochondria? It has been reported that Aβ binds to various different neuronal receptors, including but not limited to NMDA, nicotinic (α7nAChR), and p75NTR receptors. Aβ binding to NMDA receptors has been reported to increase calcium influx and potentiate NMDAR activation, but other conflicting reports have shown that Aβ oligomers depress LTP and cause progressive loss of dendritic spines associated with reduced excitatory synaptic transmission and removal of glutamate receptors from spines (Hsieh et al., 2006; Pena et al., 2006; Shankar et al., 2007). A recent study also showed that Aβ oligomers stimulate extrasynaptic NMDA (NR2B) receptor responses to inhibit LTP and activate LTD (Li et al., 2011). Conflicting reports have also shown that Aβ can act as both agonist and antagonist of α7nAChR (Dineley et al., 2001; Pettit et al., 2001). The binding of Aβ to p75NTR, especially in cholinergic neurons of the basal forebrain where this receptor is enriched, is associated with death of these neurons. In p75NTR

knockout mice, such Aβ-induced degeneration of the basal forebrain is not observed (Yaar et al., 1997). Therefore, targeting the Aβ/p75NTR interaction appears to represent a promising therapeutic strategy, especially for the cholinergic system. However in this review, we will focus on the integrin adhesion receptors, as Aβ mediates its neurotoxicity via binding to multiple integrins, which are highly enriched throughout the brain.

The integrin family mediates both cell-cell and cell-substratum adhesion by binding their extracellular ligands such as laminin and fibronectin. Integrins consist of an α and a β subunit, and each subunit has a large extracellular portion, a single transmembrane segment, and a short cytoplasmic tail (Hynes, 1992). These cytoplasmic tails bind to intracellular ligands to mediate dynamic cellular responses, such as cell adhesion, migration, neurogenesis, apoptosis, and synaptic stability. Hence, integrins provide a transmembrane link for the bidirectional transmission of signals across the plasma membrane by binding both extracellular and intracellular ligands (Zamir and Geiger, 2001). The ability to connect to the actin cytoskeleton is an important part of the adhesive function of the integrin family. Within focal adhesions, structural proteins such as vinculin and talin anchor β-integrins to the actin cytoskeleton, while signaling proteins such as focal adhesion kinase (FAK), Pyk2, Paxillin, and Src mediate downstream signaling events in a transient and controlled manner (Cabodi et al., 2010). Integrin signaling is also associated with changes in synaptic plasticity and neuronal excitability. Glutamate receptors (NMDA and AMPA receptors) are the target of integrin-dependent regulation. Interfering with integrin-dependent adhesion by antagonists or conditional deletion of α3, α5, and/or β1 integrins leads to defective LTP (Becchetti et al., 2010). Therefore, integrins represent important adhesion receptors that can regulate multiple facets of neuronal function and viability.

Multiple studies have shown that Aβ can bind to a number of different integrins, a process that can lead to both protective and neurotoxic outcomes. α5/β1-integrin has been shown to exert an anti-apoptotic role. Adhesion by α5/β1-integrin upregulates Bcl-2 and is one of the few integrins that activates the signaling protein Shc. Matter and colleagues showed that α5/β1-integrin binds to nonfibrillar but not fibrillar Aβ1-40 and reduces the formation of insoluble fibrillar Aβ via internalization of Aβ and partial degradation, an effect that is blocked by an antibody against α5-integrin. Overexpression of α5/β1-integrin protects from Aβ-induced apoptosis (Matter et al., 1998). Bozzo and colleagues demonstrated that plating of retinoic acid differentiated SH-SY5Y cells to collagen, fibronectin, or laminin reduces Aβ (25-35)-induced toxicity. Preincubation of cells with antibodies directed against β1-integrin or α1-integrin substantially enhances

Aβ toxicity when plated on collagen, presumably because disruption of integrin ligation to collagen enhances the binding of Aβ to integrins. Treatment of Aβ (25-35) also induces a strong down-regulation of β1-integrin on the cell surface while not affecting β3, α3, and α1 integrins (Bozzo et al., 2004). Therefore, integrins can modulate Aβ levels and by binding, internalization, and degradation of Aβ. In addition, the ligation of integrins to their physiological substrates can protect against Aβ-induced neurotoxicity.

On the other hand, multiple studies have shown that several integrins are also required for Aβ-induced toxicity. Integrin blocking antibodies to α1/β1 or echistatin (a highly selective and potent integrin inhibitor) blocks fibrillar Aβ-induced MAPK activation and Aβ-induced neurotoxicity in primary neuronal cultures (Anderson and Ferreira, 2004). Moreover, antibodies against αv-integrins block the inhibition in LTP induced by Aβ. In addition, a small molecule nonpeptide antagonist of αv-containing integrins (SM256) and a potent disintegrin echistatin also block Aβ-induced inhibition of LTP. Accordingly, a potent ligand of αv/β1 integrin, superfibronectin, also efficiently inhibits Aβ-induced inhibition of LTP (Wang et al., 2008). As glutamate receptors are targets of integrin-mediated regulation, it is likely that integrin-mediated alterations in glutamate receptors underlie Aβ-induced inhibition of LTP. Addition of soluble Aβ together with fibrillar Aβ dramatically enhances the meshwork of Aβ deposition on cortical neurons and induces neurotoxicity in an α2/β1 and αv/β1 integrin dependent manner (Wright et al., 2007). Antibodies against β1, α2, and αv but not α1, α3, α4, α5, α6, α9, αv/β 3, αv/β5, or β3 integrins inhibit Aβ-induced neurotoxicity in human primary neurons (Wright et al., 2007). Aβ elicits a sustained phosphorylation of pyk2-associated paxillin, which correlates with neurotoxicity. Expression of a dominant negative Pyk2 abolishes Aβ-induced neurotoxicity, and αv/β1 ligands (fibronectin, superfibronectin, and vitronectin) as well as α2/β1 ligands (collagen and laminin) all block Aβ-induced neurotoxicity, presumably via competing with Aβ binding to these receptors (Wright et al., 2007). Interestingly, both actin stabilizing compounds (jasplkinolide) and destabilizing compounds (cytochalasin D and latrunculin A) also inhibit Aβ-induced neurotoxicity, indicating that downstream actin remodeling is critical for this effect (Wright et al., 2007). Perlecan domain V, which binds to α2-integrin, competitively inhibits Aβ binding to cells as well as Aβ-induced neurotoxicity (Wright et al., 2010). α2 null cells significantly bind less Aβ than wild type cells, and Aβ binds more efficiently to cell expressing the activated form of α2-integrin (Wright et al., 2010). All of these findings taken together indicate that Aβ binding to various integrins, especially α2, β1, and αv, are required for the complement of Aβ-induced neurotoxicity. However, the normal engagement of integrins to their cognate physiological matrix substrates appears to be protective for a couple of reasons. First, the physiological substrates compete out the binding of Aβ to integrins, and second, they mediate normal and tightly regulated integrin activation and downstream signaling. Integrin activation and downstream focal adhesion signaling (FAK, Pyk2, Paxillin, and Src) induced by Aβ binding to integrins is not well characterized but appears to be a prolonged and dysregulated, unlike that seen with physiological substrates (Wright et al., 2007; Wang et al., 2008; Cabodi et al., 2010). It is likely that such aberrant transmission of focal adhesion signals and/or the breakdown of focal adhesion complexes underlie Aβ-induced neurotoxicity.

An important consideration in the role of integrins and focal adhesion signaling in AD pathogenesis is the observation that APP together with reelin physically associate with α3/β1 integrin (Hoe et al., 2009). Reelin simultaneously promotes neurite outgrowth in primary neurons as well as the nonamyloidogenic α-secretase processing of APP in an APP and α3/β1 integrin-dependent manner, thereby inhibiting the generation of Aβ (Hoe et al., 2009). The enhanced α-secretase processing of APP and reduced Aβ generation by reelin is due to increased level of surface APP by blocking APP endocytosis, since α-secretase processing of APP primarily takes place on the cell surface and endocytosis is required for Aβ generation (Koo and Squazzo, 1994; Perez et al., 1999; Hoe et al., 2009). Furthermore it is well known that the Low Density Lipoprotein Receptor-Related Protein (LRP) not only physically associates with APP but also with β-integrins (Salicioni et al., 2004). We have shown that LRP promotes APP endocytosis and Aβ generation in lipid raft microdomains (Pietrzik et al., 2002; Yoon et al., 2005; Yoon et al., 2007). In addition, LRP is required to mediate the trafficking of β-integrins to the cell surface (Salicioni et al., 2004). On the extracellular side, Aβ interacts not only with integrins but also with APP and LRP (Lu et al., 2003; Deane et al., 2009). Indeed the binding of Aβ to APP is dependent on the the Aβ domain of full-length APP on the cell surface (Lu et al., 2003). On the intracellular side, the scaffolding protein RanBP9 physically associates with APP, LRP, BACE1, and β-integrins (Denti et al., 2004; Lakshmana et al., 2009; Lakshmana et al., 2010). Such association of RanBP9 promotes APP endocytosis, interaction between APP and BACE1, and localization of APP to cholesterol-rich lipid raft microdomains, leading to increased BACE1 cleavage of APP and Aβ generation (Lakshmana et al., 2009; Lakshmana et al., 2010). These observations raise the intriguing possibility that Aβ-induced neurotoxicity as a ligand for integrins, APP, and LRP is mechanistically coupled to the generation of Aβ via endocytosis of the integrin/APP/LRP receptor complexes. Such process may displace physiological ligands (i.e. fibronectin or reelin), thereby

leading to the aberrant recruitment of intracellular factors (i.e. RanBP9) to their cytoplasmic tails to transmit neurotoxic signals (i.e. dysregulated focal adhesion signals) while simultaneously promoting Aβ generation. This vicious cycle may in part explain the progressive nature of neurodegeneration and accumulation of Aβ seen in AD.

COFILIN, A CENTRAL NEXUS OF CYTOSKELETAL CHANGES, MITOCHONDRIAL DYSFUNCTION, AND TAU PATHOLOGY IN NEURODEGENERATIVE DISEASES?

Integrins, mitochondrial dysfunction, cytoskeletal perturbations,

and tau phosphorylation are key elements of Aβ-induced neurotoxicity. However, what critical downstream intracellular factors relay the Aβ neurotoxic signals to the mitochondria, cytoskeleton, and tau? The assembly and disassembly of G-actin to F-actin is a process critical to many cellular processes, including cell motility, migration, dendritic spine morphogenesis, and membrane protein endocytosis (Bernstein and Bamburg, 2010). ADF/Cofilin, a family of actin-binding protein, is perhaps one of the key regulators of actin dynamics. Cofilin differentially modulates actin dynamics depending on the ratio of cofilin to actin. At low cofilin to actin ratios, cofilin acts to sever actin filaments, whereas at high cofilin to actin ratios, cofilin
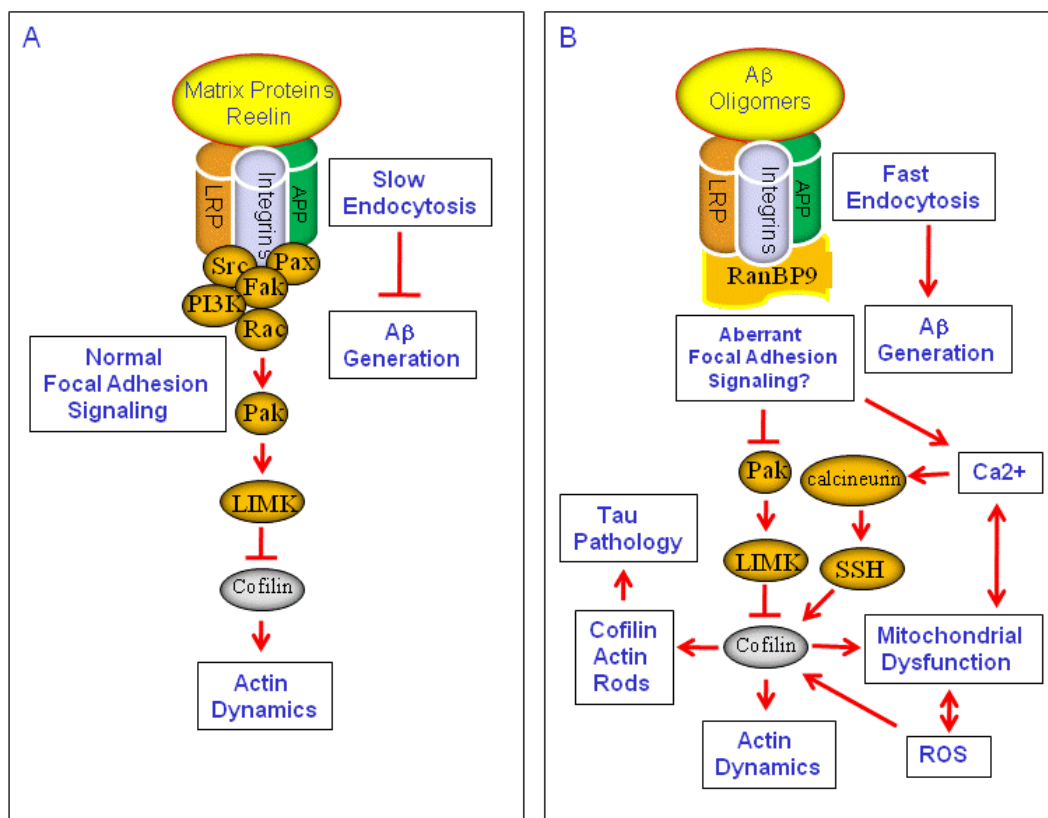

Fig. 1. Schematic of normal (A) and pathological (B) integrin-based focal adhesion signaling. (A) During normal focal adhesion signaling, the binding of integrins to their physiological ligands such as extracellular matrix proteins (i.e. fibronectin or laminin) or reelin activates various focal adhesion signaling proteins (i.e. paxillin, FAK, Src, and PI3K), leading to the activation of the Rac-PAK pathway. PAK activates LIMK which phosphorylates cofilin on serine-3. This phosphorylation inactivates cofilin, thereby transiently reducing actin dynamics. The engagement of normal physiological ligands such as reelin with the integrin/APP/LRP complexes (A) simultaneously slows down APP endocytosis, thereby reducing Aβ generation. As the binding of Aβ to various integrins is critical to transmit its neurotoxic signals, Aβ oligomers may displace normal integrin ligands, thereby transmitting defective or aberrant focal adhesion signals, eventually leading to defects in PAK activation. In appropriate or reduced PAK signaling fails to activate LIMK, thereby reducing cofilin phosphorylation. Under conditions of oxidative stress induced by Aβ oligomers or hyperphosphorylated tau, cofilin and 14-3-3ζ is oxidized leading to activation of SSH and further activation of cofilin. Oxidized and dephosphorylated cofilin translocates into the mitochondria to open up the mitochondrial permeability transition pore, an event that is required for oxidant-induced cell death. Cofilin translocation into mitochondria induces mitochondrial dysfunction, thereby further increasing ROS and calcium dysregulation. Calcium dysregulation by Aβ, tau, and/or cofilin also activates calcineurin, which then leads to SSH activation and further cofilin activation. Activated cofilin can lead to the formation of cofilin-actin rods or aggregates, which attracts hyperphosphorylated tau and promote tau pathology. At the same time, the binding of RanBP9 to APP/LRP/integrin complexes accelerates APP endocytosis and enhances Aβ generation, thereby further propagating Aβ-induced neurotoxic signaling.

nucleates actin assembly and stabilizes F-actin (Bernstein and Bamburg, 2010). The activity of cofilin is largely controlled by phosphorylation and dephosphorylation on serine-3 of cofilin by LIMK and Slingshot-1L (SSH), respectively (Bernard, 2007; Eiseler et al., 2009). Phosphorylation of cofilin by LIMK1, a downstream component of Rac-PAK signaling upon focal adhesion activation, serves to inactivate cofilin, whereas dephosphorylation of cofilin by Slingshot-1L (SSH) activates cofilin. It has also been shown that reelin, via a pathway involving Dab1, Src, and PI3K, promotes the phosphorylation of cofilin on serine-3, thereby inactivating cofilin (Chai et al., 2009). Therefore, proper relay of focal adhesion signals downstream of integrin and/or reelin activation leads to the phosphorylation and inactivation of cofilin by LIMK1, Src, and potentially other kinases, leading to reduced actin dynamics. At the same time, reelin inhibits Aβ generation by blocking APP endocytosis, a process that is both APP and integrin-dependent (Hoe et al., 2009). However, ligation of Aβ with integrins may lead to improper control of focal adhesion assembly/ signaling and dysregulation of cofilin at multiple levels and further enhance Aβ generation (Fig. 1).

Cofilin does not only play a key role in regulating actin dynamics but also mediates a critical function in oxidative stress-induced cell death via an essential mitochondrial mechanism (Chua et al., 2003; Klamt et al., 2009). Upon oxidative stress, cofilin becomes oxidized on several cysteine residues (Klamt et al., 2009). This causes cofilin to lose its affinity for actin and to translocate into the mitochondria, where it induces swelling, drop in mitochondrial membrane potential, and cytochrome c release by promoting the opening of the permeability transition pore (PTP) (Klamt et al., 2009). Interestingly, this occurs independently of Bax. In addition to cofilin oxidation, dephosphorylation (or activation) of cofilin is required for its translocation to the mitochondria and oxidant-induced apoptosis. When oxidation of cofilin is prevented, oxidant induced apoptosis is also inhibited. Furthermore knockdown of endogenous cofilin by siRNA also inhibits both oxidant and staurosporin induced apoptosis, indicating that cofilin is critical for mitochondria-mediated apoptosis (Chua et al., 2003; Klamt et al., 2009). Interestingly, inhibition of actin dynamics is associated with increased ROS production and reduced mitochondrial membrane potential (Gourlay and Ayscough, 2005), both of which can further contribute to cofilin-mediated mitochondrial dysfunction. Therefore, dysregulation of cofilin itself, which leads to inhibition of actin dynamics, can lead to increased ROS levels and mitochondrial dysfunction. Cellular stimuli that generate ROS not only oxidize cofilin to promote mitochondrial dysfunction but also activate SSH via oxidation of 14-3-3ζ, a chaperone protein that normally functions to inhibit SSH in

the nonoxidized form (Kim et al., 2009). Therefore, enhanced ROS generation by Aβ, tau, and/or other oxidative stress can dephosphorylate and activate cofilin. Another critical pathway of cofilin activation is via calcium. Calcineurin, a calcium activated phosphatase, dephosphorylates SSH, thereby promoting cofilin dephosphorylation and activation (Wang et al., 2005). Given that Aβ increases intracellular Ca^{2+}, it has been reported that Aβ also activates calcineurin and dephosphorylates cofilin via the activation of SSH (Homma et al., 2008). Furthermore, Aβ-induced dendritic spine loss is also mediated by a pathway involving calcineurin and dephosphorylation of cofilin (Shankar et al., 2007). Indeed, inhibition of calcineurin by FK506 is neuroprotective in transgenic animal models of AD (Hong et al., 2010; Rozkalne et al., 2011). Finally, it has been shown that active PAK, a downstream component of focal adhesion and Rac signaling, is severely reduced in AD brains. Aβ oligomers induce defects in PAK signaling, leading to the activation of cofilin and synaptic pathology. Overexpression of active PAK reverses these effects of Aβ oligomers (Zhao et al., 2006; Ma et al., 2008). Therefore, Aβ-induced alterations in Ca^{2+}, ROS, and focal adhesion signaling can also induce the activation of cofilin, which in turn, dysregulates cytoskeletal dynamics, mitochondria, and tau (Fig. 1B).

One of the pronounced features of multiple neurodegenerative diseases, including AD, is the accumulation of filamentous rod-like structures that contain cofilin and actin (i.e. cofilin-actin rods). These inclusions are prominently found in hippocampal and cortical tissues of AD brains and transgenic mouse models of AD but are missing in normal brains (Maloney and Bamburg, 2007). Under stressful situations such as ATP depletion, excitotoxicity, or ROS overproduction, cofilin-actin rods form in tandem array in neurites of primary neurons (Minamide et al., 2000; Whiteman et al., 2009). Treatment of primary neurons with soluble Aβ oligomers similarly induces cofilin-actin rod formation in neurites of ~20% of neurons in a time course mirroring the depletion of ATP induced by the Aβ treatment (Whiteman et al., 2009). It is not clear why Aβ does not affect all neurons in a similar fashion, but cofilin is dephosphorylated and activated only in neurons that form cofilin-actin rods. These inclusions, particularly in neurites, are thought to impair transport of proteins and organelles such as mitochondria down axons and dendrites. Moreover, a subset of cofilin-actin rods contains hyperphosphorylated tau, indicating that cofilin-actin cytoskeletal dynamics also alter tau pathology (Whiteman et al., 2009). Indeed, it has been demonstrated that transgenic mice and flies overexpressing FTDP-17 mutant tau promote F- actin assembly, and hyperphosphorylated tau co-precipitates in rods together with cofilin and actin (Fulga et al., 2007). These rod structures resemble striated neuropil threads, a

characteristic tau pathology in AD brains. Hyperphosphorylated tau loses its affinity for microtubules and mislocalizes in dendritic spines, where it functions to remove glutamate receptors and impair excitatory synaptic transmission (Hoover et al., 2010). Cofilin plays a key role in the organization of actin cytoskeleton in dendritic spines as well as Aβ-induced spine loss (Shankar et al., 2007). Thus, cofilin and tau may cooperatively control such activity and eventually precipitate in rods that contain cofilin, actin, and tau when neurons become further stressed by Aβ-induced neurotoxic signals.

Cytoskeletal and mitochondrial pathologies are not unique to AD but are also key features of both Parkinson's and Huntington's disease. Parkinson's disease (PD), the second most common neurodegenerative disease after AD, is characterized by two major pathological hallmarks: 1) selective loss of dopaminergic neurons in the substantia nigra and 2) intraneuronal inclusions termed Lewy bodies mainly composed α-synuclein (Spillantini et al., 1997). In addition to the ubiquitin-proteasome pathway, mitochondrial dysfunction plays a central role in the pathogenesis of PD. 1-methyl-4-phenyl- 1,2,3,6-tetrahydropyridine (MPTP), a mitochondrial complex 1 inhibitor, results the degeneration of nigral neurons similar to PD (Langston et al., 1984). Several toxins including MPTP and rotenone induce α-synuclein aggregation through the inhibition of mitochondrial function (Kowall et al., 2000; Lee et al., 2002b). Furthermore, iron or cytochrome c enhances the accumulation of α-synuclein (Hashimoto et al., 1999a; Hashimoto et al., 1999b). The PD-linked α-synuclein A30P mutation results in actin cytoskeletal disruption in hippocampal neurons (Sousa et al., 2009), and parkin, another PD-linked gene, reduces cofilin phosphorylation through the interaction with LIM kinase 1 (Lim et al., 2007). Knockdown of PINK1, another PD gene, leads to increased aggregation of actin and its binding with parkin while increasing cofilin phosphorylation (Kim and Son, 2010).

Huntington's disease (HD) is one of the polyglutamine disorder caused by CAG repeat expansion in the exon 1 of the huntingtin (Htt) gene. Mutant huntingtin forms aggregates in the cytoplasm and nucleus and negatively affects neuronal viability, leading to the degeneration of the striatum manifested clinically by uncontrollable involuntary movements (Spokes, 1980). Mitochondrial dysfunction has long been associated with neurodegeneration in HD. 3-Nitropropionic acid, an inhibitor of the mitochondrial citric acid cycle, leads to a selective degeneration of the striatum similar to that seen in HD (Borlongan et al., 1997). Many studies have shown that mutant Htt associates with mitochondria and causes impairments in mitochondrial function as well as inhibition of vesicular axonal trafficking and mitochondrial motility (Panov

et al., 2002; Trushina et al., 2004; Orr et al., 2008). It has also been shown that mutant Htt induces nuclear rods similar to the cofilin-actin rods seen in AD (Munsie et al., 2011).

The involvement of mitochondrial dysfunction and perturbations in the cytoskeleton and cofilin are common themes in various different neurodegenerative diseases, including AD, FTD, PD, and HD. Although Aβ accumulation is unique to AD, pathogenic intracellular proteins such as tau, α-synuclein, and huntingtin all promote cytoskeletal aberrations and mitochondrial dysfunction. Thus, whether neurotoxic signals are derived from an extracellular source (i.e. Aβ) or from within the neuron, mitochondria and cytoskeleton appear to be the primary targets of neurodegenerative processes. In AD, Aβ induces mitochondrial dysfunction, calcium dysregulation, ROS generation, and cytoskeletal aberrations via binding to integrins and hyperphosphorylation of tau. In view of the findings that cofilin regulates and is regulated by each of these key events, we hypothesize that cofilin acts as a central nexus regulating the pathogenesis and neurodegenerative changes seen in AD and related disorders. Therefore, the regulation of cofilin activity from the cell surface (i.e. Aβ binding to integrins or other receptors) or from inside neurons (i.e. ROS, Ca^{2+}, and focal adhesion signal regulation) and/or its physical interactions with the mitochondria, cytoskeleton, focal adhesion complexes, and/or tau may be attractive therapeutic targets for neurodegenerative diseases.

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