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Biochimica et Biophysica Acta 1739 (2005) 323–330

BIOCHIMICA ET BIOPHYSICA ACTA BBA

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Review

Tau and src family tyrosine kinases

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Received 27 August 2004; accepted 1 September 2004
Available online 15 September 2004

Abstract

The interaction between tau and src family non-receptor tyrosine kinases represents a new function for tau. Mediated by the proline-rich region of tau and the SH3 domain of fyn or src, this interaction has the potential to confer novel cellular activities for tau in the growth cone and in the membrane. The subsequent finding that tau is tyrosine phosphorylated has led to the observation that tau in neurofibrillary tangles is tyrosine phosphorylated. Therefore, a role for tyrosine kinases such as fyn in neuropathogenesis is predicted.

Keywords: Tau; Src; Tyrosine kinase

In 1975, tau was discovered by the Kirschner laboratory as a brain microtubule-associated protein [1]. Accordingly, many experimental findings related to tau have been interpreted in light of its function as a promoter of microtubule assembly and stability. For instance, when we reported the first sequence for a tau cDNA, the noted presence of imperfect repeat motifs in the carboxy-terminal sequence prompted us to propose that these repeated motifs would interact with the microtubule lattice [2]. Similarly, the discovery that tau was a prominent component of the neurofibrillary tangles of Alzheimer's disease [3–6] led to the hypothesis that the loss of microtubule stability would be a critical part of the neurodegenerative process in tauopathies. However, while an overwhelming number of studies have shown that tau is capable of promoting microtubule assembly in vitro and microtubule growth and stability in cells, there have been findings that suggest that tau may have functions other than the stabilization of microtubules. Two examples are the reported localizations of tau to the microtubules of the mitotic apparatus [7] and the growth cone [8–10]. The microtubules in both of these structures are highly dynamic and it is unlikely that the association of tau to these microtubules would be providing microtubule stabilization as envisioned for tau's localization in the axon.

Perhaps the idea that tau should have additional functions is an obvious one. After all, when the sequence for the brain microtubule-associated protein MAP2 was reported, it was found to have significant homology to tau in its carboxy-terminal domain, with similar imperfect repeat motifs [11]. Therefore, the molecular basis for tau's ability to stabilize microtubules was certainly not unique. The idea that tau might have additional functions was further underlined by antisense experiments performed on primary neuronal cultures. When cultures were grown in the presence of tau antisense, exploratory neurites formed but subsequent axonal development was inhibited [12]. In the presence of MAP2 antisense, exploratory neurites were inhibited, indicating that MAP2, rather than tau, was more critical towards initiating the growth of neurites [13]. At the time, the interpretation of the tau antisense experiment was that tau was required for the conversion phase during which stable microtubules became bundled within the axon. But these findings raised the following questions. In tau antisense treated neurons, why was MAP2, which was also capable of bundling microtubules, unable to facilitate axonal development? Similarly, in the MAP2 antisense treated neurons, why was tau unable to promote the growth of

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doi:10.1016/j.bbadis.2004.09.002

exploratory neurites? These questions, which remain largely unanswered, suggest that these MAPs serve additional functions other than microtubule stabilization during neuronal development.

Other analyses performed using tau depletion strategies have also questioned tau's indispensability as a microtubule-associated protein. The first genetically engineered tau-deficient mouse was described as having altered microtubule distributions in small caliber axons with other characteristics being otherwise normal [14]. This relatively unimpressive phenotype challenged the idea that tau was critical for axonal development. Consistent with these results were those obtained by injection of tau antibodies into cultured sympathetic neurons, showing no effect on axonal growth [15]. Additional insights were provided by the report that primary neuron cultures grown on laminin, as opposed to polylysine, did not require tau for axonal development provided MAP1B was present [16]. Moreover, the tau-deficient mouse had been found to have an up-regulation of MAP1A, another microtubule associated protein [14], and when the tau-deficient mouse was crossed with a MAP1B deficient mouse, the double "knockout" mouse exhibited a number of developmental defects not present in either of the single "knockout" parents [17]. Put together, these findings suggest that other microtubule-associated proteins can compensate for the loss of tau during axonal development and provide us with glimpses into the resiliency of neurons.

Yet other studies did find alterations that correlated with tau depletion. In tau antisense treated neurons, an examination of the growth cones of the exploratory neurites revealed changes in the actin distribution and reductions in growth cone size [8]. In fact, acute inactivation of tau in the chick DRG growth cone achieved by micro-scale chromophore assisted laser inactivation showed that there was a local decrease in the lamellipodial size in the region of inactivation as well as a decrease in the neurite extension rate [18]. In addition, primary hippocampal cultures derived from a tau-deficient mouse showed a delay of neuronal maturation, with a slowed rate of axonal extension [19] while a tau-deficient mouse was reported to have motor and behavioral deficits, consistent with a requirement for tau in development [20]. Based on these findings, one could speculate that tau has a role in the forward movement of the growth cone. Moreover, the finding that neurite outgrowth required a phosphorylated form of tau that associated poorly with microtubules [21] supported the notion that in growth cones, tau would have a function distinct from microtubule stabilization. This is consistent with the observation that in growth cones, tau's association to the microtubules was more labile relative to that of other neuronal MAPs, as concluded from the experimental conditions necessary to capture the association [9]. These findings raise the possibility that in the growth cone tau would bind to dynamic microtubules with a lower affinity while simultaneously carrying out some other function. Dynamic microtubules were required for the forward movement of the growth cone [22,23] and when microtubule dynamics were reduced, growth cone lamellae were compromised [24]. If tau has a role in tethering growth cone lamellae to dynamic microtubules, its depletion would affect growth cone lamellipodia, thereby affecting growth cone advance.

The unique role of tau in axonal development, suggesting that tau would have other functions besides microtubule stabilization, motivated our laboratory to search for new functions for tau. Our first approach focused on the amino terminus of tau. This domain of tau did not associate with microtubules and by expressing this tau fragment in PC12 cells, we found that the amino terminus of tau associated with the plasma membrane [10]. Moreover, the over-expression of the amino terminus of tau inhibited NGF induced process outgrowth in PC12. These findings suggested that the membrane association of tau had a role in neurite outgrowth. If tau were responsible for stabilizing microtubules close to the membrane, this could explain the morphological change noted in the tau-deficient mouse since small caliber axons would have proportionately more microtubules close to the membrane than other axons.

In a separate study, we tested tau for interaction with SH3 (src homology 3) domains. These domains are thought to mediate protein–protein interactions and were first identified in the non-receptor tyrosine kinase src (reviewed in Refs. [25,26]). The rationale for testing tau stemmed from the report that SH3 domains often recognize proline-rich areas similar to those found upstream of tau's microtubule binding repeat domain [27,28]. In addition, an SH3 domain had been identified in spectrin [29], a membrane associated protein reported to interact with tau [30]. By testing a panel of SH3 domains, we found that although tau did not bind to the spectrin SH3 domain, it bound to those from the src family non-receptor tyrosine kinases, src, fyn and lck [31]. Mapping the site of interaction on tau identified the PXXP sequence at ^{233}pro-lys-ser-pro as the site employed for interaction with the fyn SH3 domain. Co-immunoprecipitation experiments carried out in SH-SY5Y human neuroblastoma cells indicated that a small fraction of the tau and fyn associated with each other, suggesting a highly regulated interaction. Phosphorylation is likely to regulate the interaction, as peptide binding data has shown that phosphorylation at thr231 decreased the binding [32].

The functional implications for SH3 domain interactions include directing substrates to the catalytic site of the kinase [33] and the intracellular targeting of proteins [34]. To determine if tau acted as a substrate for fyn, we performed co-transfections of tau and fyn in COS cells and also tested tau for in vitro phosphorylation by fyn [35]. Both assays resulted in the tyrosine phosphorylation of tau and, using site-directed mutagenesis, tyr18 was identified as the sole tyrosine in human tau phosphorylated by fyn in transfected cells [35]. Phospho-specific antibodies raised against this site indicated that in mouse, tyr18 was phosphorylated early in neuronal development but was not phosphorylated in the

adult. Tyrosine phosphorylated tau has also been reported in human fetal brain by Williamson et al. [36]. These investigators have similarly reported the tyrosine phosphorylation of tau by lck in vitro and have identified tyr29 as the site modified. In addition, not surprisingly, we have found that src will phosphorylate tau; tyr18 as well as other site(s) were modified (our unpublished data).

In contrast to the phosphorylation of tau on some serines and threonines, the tyr18 modification did not impact on its microtubule association properties [35]. On the other hand, unlike serine and threonine phosphorylation, which account for greater than 99% of protein phosphorylation in cells, tyrosine phosphorylation is relatively rare. Moreover, tyrosine phosphorylation is closely associated with highly regulated and dynamic signal transduction processes. In neuronal cells, the importance of tyrosine phosphorylation in growth cone function is well established [37,38]. Growth cones contain src and fyn [39,40] and neurons cultured from mice deficient in either src or fyn were defective in neurite outgrowth in a substrate-dependent manner [41,42]. Using inhibitors of src family tyrosine kinases (PP1 and PP2) has allowed these kinases to be identified as part of the signaling pathway connecting cell adhesion molecules and the cytoskeleton during axon guidance in aplysia [43]. The tyrosine phosphorylation of tau leads us to speculate that tau will have a role in neuronal signal transduction.

As a functional implication of an SH3 domain interaction, the intracellular targeting of proteins could include the localization of tau to the plasma membrane. Since src family non-receptor tyrosine kinases associate with lipid rafts through their amino-terminal modifications of myristoylation and palmitoylation [44,45], it is conceivable that the SH3 domain interaction has a role in directing tau to lipid rafts. Lipid rafts are microdomains of plasma membrane that are enriched in cholesterol and sphingolipids and are thought to serve as platforms for signal transduction [46,47]. In oligodendrocytes, tau–fyn complexes have been found in lipid rafts and when the lipid rafts were abolished through the inhibition of sphingolipid synthesis by fumonisin B1, process outgrowth was inhibited [48]. This argued for a role for lipid rafts and the fyn–tau interaction in process outgrowth. However, it remains to be proven that the fyn–tau interaction is required for the lipid raft localization of tau.

We had previously shown that in PC12 cells, tau can associate with the plasma membrane [10]. We have also found tau in the lipid raft fractions purified from PC12 and SH-SY5Y cells (our unpublished data). While the plasma membrane would certainly include the lipid raft fraction, lipid rafts comprise a relatively minor fraction of the plasma membrane. Hence, tau data obtained from a total plasma membrane preparation may not necessarily relate to the lipid raft tau since there may exist a large plasma membrane (non-raft) tau population that differs from lipid raft tau with respect to phosphorylation state and molecular associations.

The requirement for lipid rafts in growth cone motility has been supported by studies performed in primary neuronal culture using either pharmacological treatments to disrupt lipid rafts or chromophore-assisted laser inactivation of gangliosides, a primary lipid raft component [49]. Similarly, disruption of lipid rafts blocked growth cone guidance and responses to extracellular signals [50], in addition to inhibiting the development of neuronal polarity in primary hippocampal neurons [51]. It is tempting to consider the possibility that the importance of tau in axonal development and growth cone function will be related to its localization in lipid rafts. Tau in the lipid raft fraction was phosphorylated on ser262 (our unpublished data), suggesting that tau in lipid rafts will associate poorly with microtubules. The lipid raft tau was phosphorylated on tyr18 also (our unpublished data), perhaps allowing for interactions with downstream signaling proteins containing SH2 or PTB domains. A role for tau in signal transduction might explain the presence of tau in a variety of non-neuronal tissues such as lung, kidney, skeletal muscle and heart [52,53]. Interestingly, when brain mRNA from the tau-deficient mouse was analyzed by microarray to profile gene expression, the largest change observed was an increase in the expression of c-fos [54]. Since c-fos is a transcription factor that is induced as part of the signal transduction pathways activated during the early response to growth factors, these findings suggest that depletion of tau impacts on signal transduction.

The connection between tau and src family tyrosine kinases led us to ask if any evidence existed to support a role for these kinases in Alzheimer's disease. Interestingly, more than a decade ago Wood and co-workers reported that neuritic plaques and dystrophic neurites in AD brain contained phosphotyrosine [55] and that fyn was up-regulated in a subset of neurons that also contained abnormally phosphorylated tau [56]. Studies that have provided additional evidence for tyrosine phosphorylation in Alzheimer's disease have used either cultured cell systems which have been exposed to Aβ or immunocytochemical approaches to link tyrosine phosphorylation to neuropathology.

In PC12 cells, Aβ exposure induced higher levels of tyrosine phosphorylated proteins [57]; this finding has been replicated in other neuronal cell systems, including primary neuronal cultures [36,58–60]. Several laboratories have reported increased levels of tyrosine phosphorylated kinases in response to Aβ; these include focal adhesion kinase [36,61], phosphatidylinositol 3-kinase [62], and MAP kinase [63]. Focal adhesion kinase is a known substrate for fyn and Aβ treatment was reported to up-regulate the association between focal adhesion kinase and fyn [36,64], with Aβ induced phosphorylation of focal adhesion kinase being subject to inhibition by the src/fyn inhibitor PP2 [36]. Aβ has also induced increases in the tyrosine phosphorylation of paxillin [58], another known substrate for fyn. The involvement of fyn in Aβ initiated signaling cascades in neurodegeneration was first suggested by the finding that neurons from mice deficient in fyn were protected against

Aβ induced neurotoxicity [65]. Fyn has also been implicated in an Aβ initiated pro-inflammatory signaling pathway in microglial cells [60] as well as a pathway for Aβ-enhanced glutamate neurotoxicity involving the α7 nicotinic receptor and phosphatidylinositol 3-kinase [66]. An interaction between Aβ and integrins [67–70] would also invoke established signaling pathways that include fyn [71,72]. In addition, fyn can activate GSK3β [73] and cdk5 [74], both known to phosphorylate tau at disease-associated epitopes.

The activation of fyn during neuropathogenesis is consistent with immunocytochemical studies in AD brain reporting that clustered integrin receptors, tyrosine phosphorylated paxillin, and tyrosine phosphorylated focal adhesion kinase co-localized with tau in dystrophic neurites [58]. The subcellular localization of fyn in AD brain has been recently examined in a preliminary study showing that during the progression of Alzheimer's disease, fyn shifted from a synaptic location to the cell body [75]. Extending the studies performed on human tissue have been those performed in mouse models. In a mouse expressing a mutant form of the amyloid precursor protein (Tg2576), fyn was found in dystrophic neurites in senile plaque cores co-localizing with activated GSK3β [76]. The functional consequences of fyn in the context of Alzheimer's disease, beyond the phosphorylation and activation of numerous proteins, were probed using mouse models that manipulated APP/Aβ levels and fyn levels. By using low and high APP producing mice and fyn overexpressing or deficient mice, fyn overexpression was found to exacerbate the synaptic defects exhibited by the APP mice while fyn depletion reduced the synaptic defects [77]. In addition, fyn overexpression increased premature death in APP mice while fyn depletion decreased the mortality of APP mice [77]. These data support a role for fyn in the synaptotoxicity and neurotoxicity mediated by APP and/or Aβ.

Analogous to the finding that neurons from fyn-deficient mice were protected against Aβ induced neurotoxcity, Rapoport et al. [78] have reported that neurons from tau-deficient mice were similarly protected. Thus, it is tempting to speculate that the fyn–tau interaction would have a role in the neurodegenerative process, perhaps separate from tau's polymerization into filaments. If so, one would expect tau to be phosphorylated on tyr18 during the neuropathogenic process. Tau was tyrosine phosphorylated in response to Aβ [36] and, using phospho-specific antibodies directed against phospho-tyr18, we found that tau was phosphorylated on tyr18 in neurofiibrillary tangles and in isolated paired helical filament preparations as well [35]. The possible involvement of the fyn–tau interaction in disease is consistent with the presence of phosphorylated tau, as detected by PHF-1, in the lipid rafts isolated from AD but not control human brain [79]. Similar analysis performed in the Tg2576 transgenic mouse model confirmed an age-dependent accumulation of tau in lipid rafts [79]. Thus, the known localization of fyn, and now tau, to lipid rafts coincides with a series of emerging studies implicating lipid rafts in the pathogenesis of Alzheimer's disease. Lipid raft preparations have been found to contain Aβ, presenilin, β-secretase, and amyloid precursor protein [80–83]. Furthermore, the disruption of lipid rafts decreased the production of Aβ [84]. As lipid rafts are thought of as membrane microdomains that facilitate signal transduction by enabling protein–protein interactions (reviewed in Refs. [47,85,86], lipid rafts may have a role in Aβ activated pathways. These pathways would activate fyn, leading to the phosphorylation and conformational changes in tau that would initiate tau fibrillarization. Interestingly, the association of tau filaments with membranes in the diseased brain has been viewed by electron microscopy [87].

On a separate note, if fyn were to become activated during neurodegeneration, a mitogenic or cell proliferative response might ensue. Src family kinases are required for growth factor activated signal transduction pathways that lead to DNA synthesis and cell proliferation (reviewed in Ref. [88]) and the activation of src during mitosis is also well established. Evidence for the involvement of fyn in cell cycle-related processes has been obtained in various contexts. Expression of kinase-inactive fyn inhibited the PDGF-induced cell proliferative response in fibroblasts [89] and lymphocytes from fyn-deficient mice were blocked in cytokinesis [90]. Mouse oocytes, arrested in metaphase, required fyn for activation and cell cycle progression [91]. Moreover, constitutively active src was the original oncogene identified from Rous sarcoma virus and will transform cells to become cancerous. Interestingly, human prostate cancer cells have tyrosine phosphorylated tau [92] and the oncogene v-fms, a src family tyrosine kinase expressed in human histiocytic lymphoma cells, can kinase tau in vitro [93]. These findings from non-neuronal cells may serve to correlate the tyrosine phosphorylation of tau to cell signaling pathways where src family tyrosine kinases have been activated and a mitogenic response has been elicited.

The relationship between the loss of cell cycle control and neurodegeneration has received much support (reviewed by Refs. [94–97]). Initiating this line of thought were early studies that, based on the phosphorylation state of tau, proposed that degenerating neurons recapitulated fetal development [98–100]. The notion that neurodegenerating cells had compromised their post-mitotic state was more directly put forth when the phospho-epitopes on neurofibrillary tangles were found to be characteristic of those found in mitotic cells [101–103]. This suggested that neurodegenerating cells and mitotic cells shared the same collection of activated kinases and that the neurodegenerating cell might be making an attempt to undergo mitosis and to reenter the cell cycle. This hypothesis has been bolstered by investigations that probe the cell cycle state of the adult brain. In AD brain, markers indicative of an activated cell cycle have been observed [104–106] and in situ hybridization data have revealed a significant number of neurons that were tetraploid [107], directly showing that DNA replication had taken place. Furthermore, activated cell

cycle markers were present even in brains from patients that had mild cognitive impairment [108]. These data suggest that as a mechanism for neurodegeneration, the loss of cell cycle control may occur early in the disease process and may not be specific to Alzheimer's disease.

Insights on the possible impact of inappropriately activated src family tyrosine kinases in the brain have been provided by a transgenic mouse model where a viral oncoprotein was expressed in post-mitotic neurons [109]. This manipulation led to neuronal cell loss with further analysis showing that the neurons had undergone DNA synthesis as well as DNA fragmentation [110]. Thus, if Aβ activates fyn, neuronal cell loss could ensue as a result of cell cycle related mechanisms. In addition, since the activity of src family non-receptor tyrosine kinases can be potentiated by the binding of SH3 ligands [111, 112], the fyn–tau interaction could have a role in transducing the signals involved. Moreover, in FTDP-17 diseases, mutated tau protein may help initiate the signals that lead to the loss of cell cycle control. It will be important to determine if a loss of cell cycle control occurs in the FTDP-17 diseases.

In summary, the interaction between tau and fyn has enlarged the perceived role of tau in neuronal cell function. The tyrosine phosphorylation of tau and its localization to lipid rafts suggest possible roles in signal transduction for tau. Insights into new functions for tau will help elucidate its unique role in neuronal cell polarity and neurodegenerative disease. At the same time, the role of fyn in neurodegenerative disease needs further investigation.

Acknowledgement

The author wishes to acknowledge Dr. Vandana Sharma for valuable comments on the manuscript. GL is supported by the NIA and the NINDS.

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