Transglutaminase Is Linked to Neurodegenerative Diseases

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Abstract

Transglutaminase catalyzes a covalent bond between peptide-bound glutamine residues and either lysine-bound peptide residues or mono- or polyamines. Multiple lines of evidence suggest that transglutaminase is involved in neurodegenerative diseases including Alzheimer disease, progressive supranuclear palsy, Huntington disease (HD), and Parkinson disease. In all of the neurodegenerative diseases examined to date, transglutaminase enzyme activity is upregulated in selectively vulnerable brain regions, transglutaminase proteins are associated with inclusion bodies characteristic of the diseases, and prominent proteins in the inclusion bodies are modified by transglutaminase enzymes. These prominent proteins in the inclusion bodies, including tau, α-synuclein, and huntingtin protein, are modified by transglutaminase in vitro and α-synuclein and huntingtin protein are modified in cells in culture. Similar changes in transglutaminase and transglutaminase-modified proteins are replicated in transgenic mouse models of the neurodegenerative diseases, including Huntington disease and progressive supranuclear palsy. Lastly, inhibition of transglutaminase either via drug treatments or molecular approaches is beneficial for the treatment of HD transgenic mice but has yet to be explored for the other neurodegenerative diseases. Further research is needed to determine the specific role(s) that transglutaminase plays in the pathophysiology of neurodegenerative diseases with possible implications for transglutaminase as a therapeutic target.

Key Words: Alzheimer disease, Huntington disease, Progressive supranuclear palsy, Parkinson disease, Transglutaminase.

INTRODUCTION

The involvement of transglutaminases in the pathophysiology of a neurodegenerative disease, namely Alzheimer disease (AD), was first suggested more than two decades ago by Dennis Selkoe, Carmela Abraham, and colleagues in their investigations on neurofibrillary tangles (1, 2). Their studies demonstrated the presence of transglutaminases in postmortem human brain of normal individuals and in those with AD. Transglutaminases are inducible enzymes, with increased activity during development, terminal differentiation, and apoptosis (3). Increases in transglutaminase-catalyzed ε-(γ-glutamyl) lysine bonds are found in various pathologic tissues such as atherosclerotic plaques and tumors and in lens tissue in cataract formation (4, 5) as well as in several neurodegenerative diseases (6–8).

Transglutaminases (EC 2.3.2.13) are a family of calcium-activated enzymes that catalyze the transamidation of glutamine-containing peptides and proteins by primary amine nucleophiles such as polyamines or small amines such as serotonin, spermine, or spermidine (9). Transamidation increases the stability of the protein substrates (10). Transglutaminase also catalyzes covalent ε-(γ-glutamyl) lysine cross-links between peptide-bound lysine and peptide-bound glutamine residues of substrate proteins (for reviews, see References 11, 12). This type of covalent bond can cross-link proteins into stable, rigid, insoluble complexes (13), reminiscent of inclusion bodies seen in neurodegenerative diseases. Transglutaminases can act in one of two ways to form stable polymers (14). Transglutaminase can direct the de novo formation of small oligomers and polymers by cross-linking substrate proteins such as in the formation of copulatory plugs. Alternatively, transglutaminase can “spot-weld” preformed oligomers and polymers reversibly assembled via noncovalent bonds, resulting in the stabilization of the polymer. The transglutaminase, factor XIIIa, functions in this way to stabilize the formation of blood clots. Based on data demonstrating the assembly of dynamic tau filaments in vitro, transglutaminase-catalyzed cross-links may stabilize assembled tau oligomers in AD.

TRANSGLUTAMINASE-CATALYZED BONDS IN INCLUSION BODIES

The transglutaminase-catalyzed bond has been shown to be associated with intracellular inclusions and the proteins contained within those inclusions in several neurodegenerative diseases, suggesting that it is a general mechanism or common pathway involved in the pathophysiology of these diseases. In AD and progressive supranuclear palsy (PSP), one of the major inclusion bodies is neurofibrillary tangles, which label with the transglutaminase-catalyzed cross-link directed antibodies (15–17). Purified straight and paired helical filaments from AD and PSP tissue contain transglutaminase cross-linked tau protein as demonstrated by immunoprecipitation and Western blots (16, 18). Furthermore, the levels of the transglutaminase-catalyzed bonds were found to be increased with age and more than threefold higher in AD compared with age-matched controls in selectively vulnerable brain regions, including the...
hippocampus and frontal cortex, as measured by an ELISA method (19). Isolation of ubiquitin-positive particles from the insoluble fraction of AD brain contained tau proteins and were found by high pressure liquid chromatography analysis to contain a number of peptide sequences that were cross-linked by transglutaminase (19). Importantly, in AD, cross-linking of tau filaments occurs before the presence of microscopically detectable neurofibrillary tangles, suggesting that transglutaminase plays a role in the formation or stabilization of small tau oligomers or monomers (18, 20).

Similar evidence suggests that transglutaminase-catalyzed bonds are associated with Lewy bodies, the major inclusion body in Parkinson disease (PD). By use of immunohistochemistry, α-synuclein-labeled Lewy bodies co-label with antibodies directed against the transglutaminase-catalyzed bond in PD cases and cases of dementia with Lewy bodies (17, 21, 22). The transglutaminase-catalyzed cross-link bond comigrates on Western blots with α-synuclein from PD substantia nigra (17, 22).

In Huntington disease (HD), perinuclear cytoplasmic inclusions and intranuclear inclusions of mutant huntingtin are prominent neuropathologic hallmarks (23–27). Transglutaminase-catalyzed cross-links are present in neuronal nuclei in selectively vulnerable brain regions in HD and the cross-links colocalize with the huntingtin protein in the inclusions (28, 29). HD and control brain tissue from the superior frontal gyrus were analyzed at the confocal level with double label immunofluorescence using an antibody specific for the e-(γ-glutamyl) lysine cross-link catalyzed by transglutaminase and an antibody specific for the amino terminus of the huntingtin protein in conjunction with 4',6-diamidino-2-phenylindole, a dye that specifically labels nuclei (29). In the HD cases, 4',6-diamidino-2-phenylindole-labeled nuclei contain aggregates of huntingtin that colocalized with e-(γ-glutamyl) lysine cross-links.

**IN VITRO CROSS-LINKING AND CROSS-LINKING IN CELL CULTURE**

Several major proteins that comprise the inclusion bodies can be cross-linked by transglutaminase in vitro. Tau is an excellent substrate for e-(γ-glutamyl) lysine cross-linking by transglutaminase 2 in vitro (18, 30). Amine donor and acceptor sites on human tau 23 and 40 (using the Goedert numbering system) were identified in an in vitro study (31). Not all of the glutamine and lysine residues in tau could act as acceptor or donor sites. However, many of the amine acceptor and donor sites on human tau are located in and adjacent to the microtubule binding repeat region, a region of the tau molecule implicated in the etiology of tauopathies based on the abundance of disease-associated mutations in the region. Tau protein is highly phosphorylated in AD, PSP, and other tauopathies, especially tau in the paired helical filaments and neurofibrillary tangles. Transglutaminase can cross-link phosphorylated and nonphosphorylated tau protein (32). Furthermore, transglutaminase can cross-link tau into straight and paired helical filaments (33), bundles of 10-nm filaments, and can induce Alz50 immunoreactivity (which recognizes a disease-related epitope in tau) in vitro (32). However, there are no reports of transglutaminase-catalyzed cross-linking of tau protein in cells in culture. Transglutaminase may be capable of stabilizing (i.e. “spot-welding”) preformed tau oligomers rather than inducing oligomer formation in cells in culture.

In vitro, transglutaminase 2 is also capable of cross-linking α-synuclein, a major component of Lewy bodies in PD (21). In the in vitro cross-linking of α-synuclein results in the formation of high molecular weight polymers. Similarly, in cells transfected with α-synuclein and transglutaminase 2, Triton X-100-insoluble high molecular weight aggregates are formed (21).

HD is an autosomal dominant neurodegenerative disorder caused by an unstable CAG triplet repeat expansion in the open reading frame of the first exon coding for a polyglutamine stretch in the amino terminus of the huntingtin protein (34). Normal individuals have between 11 and 34 CAG repeats in the huntingtin gene. However, patients with HD have 36 to 150 CAG repeats in the huntingtin gene coding for an expanded polyglutamine stretch (34–36). In vitro and in cells in culture, huntingtin protein with a expanded polyglutamine repeat region is an excellent substrate for transglutaminase (8, 37, 38) whereby the rate of the reaction increases over an order of magnitude with 40 or more polyglutamine repeats (38). Furthermore, transglutaminase catalyzes the formation of formic acid soluble aggregates of mutant huntingtin protein in cells in culture (29). However, in transfected cells, the predominant form of huntingtin protein that contains the transglutaminase-catalyzed bond is a monomer (39).

**TRANSGLUTAMINASE ISOENZYMES**

To date, nine transglutaminase isoenzymes coded for by distinct genes have been identified in mammals. Transglutaminase isoenzymes are differentially regulated and show different substrate specificity (40). Transglutaminases are found in a variety of mammalian tissues including the central and peripheral nervous system. The most well-characterized isoenzymes expressed in human brain are factor XIIIa, transglutaminase 2 (also known as tissue transglutaminase), and transglutaminase 1 and 3 (7).

In rat brain, two alternatively spliced isoforms of transglutaminase 2 have been described, a short and a long form (40, 41). The mRNA expression of the two transglutaminase 2 isoforms in human brain has been reported in AD cortex and in PSP brain but not in control cases (6, 42). Interestingly, the short isoform of transglutaminase 2 mRNA is not expressed in HD in a selectively vulnerable brain region; rather, the long form of transglutaminase is increased (39). A shorter human transglutaminase 2 isoform has also been identified in human erythroblukemia cells. This shorter human transglutaminase 2 is similar to the shorter rat isoform in that the shorter isoform contains a different carboxyl terminus than the longer isoform. Whereas the long form is constitutively expressed, the short form is induced by cytokines, such as interleukin-1β (41). Because cytokines, including interleukin-1β, are upregulated in AD (43), interleukin-1β could underlie the increased expression of this short transglutaminase form in AD. There are also reports of increased levels of mRNA coding for
transglutaminase 1 and 2 in AD and PSP (6, 7). It is not known whether there is an accompanying increase in protein expression of transglutaminase 1 in AD (44) or an increase in protein levels of the short isoform of transglutaminase 2 in either AD or PSP. There is an increase in transglutaminase 2 mRNA in substantia nigra of PD cases compared with age-matched control cases (17), but the expression of the short transglutaminase 2 isoform has not been examined.

Transglutaminase proteins are found in neurons in brain regions with inclusion bodies and in the inclusion bodies themselves. Transglutaminases are present in human hippocampal neurons and in AD brain colocalize with neurofibrillary tangles in hippocampal neurons (45). The immunolabeling with transglutaminase antibodies was more intense in AD hippocampus compared with age-matched controls (45). Similarly, transglutaminase 2 has been colocalized with mutant huntingtin in intranuclear inclusions in HD brain (29). Transglutaminase 2 expression was detected in neurons with Lewy bodies in PD substantia nigra, but not in controls (17, 22).

**TRANSGLUTAMINASE ACTIVITY**

The activity of transglutaminase enzymes is highly regulated. Transglutaminase activity is increased in pathologically vulnerable brain regions in the neurodegenerative diseases thus far examined. In PD, transglutaminase activity is higher in the globus pallidus and pons, two selectively vulnerable brain regions, compared with controls, whereas activity in PSP occipital cortex, a neuropathologically spared region, is comparable to control levels (6). In HD, neuronal nuclei contain increased transglutaminase protein levels and higher enzymatic activity compared with control cases (8, 46). Similarly, the activity of transglutaminase is higher in the cortex in AD cases compared with controls (7, 47).

**REGULATION OF TRANSGLUTAMINASE**

The regulation of transglutaminase has been examined in several studies. The human transglutaminase 2 gene has been analyzed: the gene contains 13 exons and 12 introns, and exons 6 and 10 are alternatively spliced (48). Analysis of the 5 upstream region resulted in the identification of several putative promoter sites including three potential SP1 sites and two potential AP2 sites. Transglutaminase 2 also functions in transmembrane signaling (49, 50). Transglutaminase transmits signals from α1-adrenoreceptors to phospholipase C-γ1 in human heart. The two functions of transglutaminase 2 are antagonistic: at high calcium concentrations the cross-linking function is active whereas at low calcium concentrations GTP binding occurs. Similarly, at high concentrations of GTP, the cross-linking function is inhibited.

**Calcium Regulates Transglutaminase Activity**

Transglutaminase enzymes are calcium-dependent zymogens containing a cysteine in their active site that is unmasked only in the presence of calcium; thus, calcium is their universal activator (11). A loss of calcium homeostasis is a common characteristic of neurodegenerative diseases (51), and the regulation of intracellular calcium is altered and levels are increased in aging (52).

**Calmodulin Regulates Transglutaminase Activity**

Calmodulin is a 17-kDa protein, which upon calcium binding activates a host of enzymes (53). Calmodulin activates transglutaminase in systems such as the human erythrocyte cytoskeleton (54), human platelets, and chicken gizzard (55). We observed colocalization of calmodulin with huntingtin, transglutaminase 2, and the transglutaminase-catalyzed bonds within nuclei in HD cortex (56). Mutant huntingtin associates with calmodulin as demonstrated by affinity purification (57) and immunoprecipitation approaches (56). The calmodulin inhibitor w5-hydrochloride prevents cross-linking of huntingtin in cells that are cotransfected with the amino terminal of huntingtin protein with an expanded polyglutamine domain and transglutaminase 2 (56).

**Transglutaminase Upregulation in Transgenic Mouse Models of Neurodegenerative Diseases**

The involvement of transglutaminase in transgenic mouse models of neurodegenerative diseases has been studied in models of HD and tauopathy but not PD or AD (or more specifically in models of the amyloid pathology in AD). Our data demonstrate that transglutaminase-catalyzed cross-links are present in the neurofibrillary tangles and paired helical filament tau from P301L tau transgenic mice but not nontransgenic control mice and transglutaminase activity is higher in the P301L tau transgenic mice (58).

The contribution of transglutaminase to the pathology in HD mouse models has been studied more extensively. Transglutaminase activity is higher in two HD transgenic mouse models, R6/2 and YAC128 mice (59, 60). Transglutaminase 2 has been knocked out in two different transgenic mouse models of HD (61, 62). R6/1 and R6/2 HD transgenic mice overexpress exon 1 of the human huntingtin gene with an expanded CAG repeat (expressing 115 and 150 repeats, respectively) driven by the human huntingtin promoter. These mice display age-related motor dysfunction, weight loss, neuropathology, and a decreased life span reminiscent of the human disease. Knockout of transglutaminase 2 in R6/1 and R6/2 HD transgenic mice increases survival and prolongs motor function. Interestingly, ablation of transglutaminase 2 increases aggregate formation in these transgenic mice, suggesting that transglutaminase does not contribute to the formation of inclusions in HD but contributes to the pathophysiology by some other mechanism. Our results demonstrate that transglutaminase predominantly modifies monomeric huntingtin, suggesting that transglutaminase catalyzes binding to a monamine or polyamine (39), consistent with the findings that transglutaminase does not increase huntingtin inclusions in HD transgenic mice.

Cystamine is a transglutaminase inhibitor that has been tested in HD transgenic mice (59, 60, 63). Cystamine reduced transglutaminase activity, improved weight loss, reduced motor dysfunction, and increased survival of the R6/2 HD transgenic mice compared with saline-treated and untreated littermates (60, 63). Treatment with cystamine starting at
Excitotoxic damage induced by glutamate exposure and after glutaminase activity as well as a second phase in which nodose ganglia, also results in a rapid increase in transglutaminase activity. Other peripheral nerves, such as the facial nucleus and increased in a calcium-dependent manner. Axotomy to toxins or ischemia, has been associated with neurodegenerative diseases and greatly facilitate detection of the isopeptide bond over previous methods such as high pressure liquid chromatography. Although the effectiveness of 81D4 on Western blots has been questioned, this antibody is specific for the transglutaminase-catalyzed GGEL bond when used for immunoprecipitation and ELISA methods. In vitro cross-linking of proteins and cross-linking in transfected cells have demonstrated the ability of this antibody to specifically and quantitatively detect the transglutaminase-catalyzed GGEL bond.

**DETECTION OF THE TRANSGLUTAMINASE-CATALYZED BOND**

Antibodies directed against the ε-(γ-glutamyl) lysine bonds (often abbreviated GGEL for “gamma glutamyl epsilon lysine”) such as 81D4 from Covance Laboratories (Lyon, France) are a useful tool in research on neurodegenerative diseases and greatly facilitate detection of the isopeptide bond over previous methods such as high pressure liquid chromatography. Although the effectiveness of 81D4 on Western blots has been questioned, this antibody is specific for the transglutaminase-catalyzed GGEL bond when used for immunoprecipitation and ELISA methods. In vitro cross-linking of proteins and cross-linking in transfected cells have demonstrated the ability of this antibody to specifically and quantitatively detect the transglutaminase-catalyzed GGEL bond.

**ACTIONS OF TRANSGLUTAMINASE IN NEURODEGENERATIVE DISEASES**

Although mutations in tau can result in the formation of tau filaments and neurodegeneration, the mechanisms underlying the formation of tau filaments in AD are less clear because tau mutations are not associated with AD. Although the amyloid hypothesis is continually gaining support from new studies, the connection between Aβ and tau pathology remains tenuous. Aβ can increase enzymes involved in the phosphorylation of tau, but tau phosphorylation is not sufficient to result in formation of tau filaments. One of the mechanisms by which tau protein could be perturbed in AD is via increases in cytokine activity and intracellular levels of calcium caused by Aβ that could increase transglutaminase expression and activity, respectively. The increase in transglutaminase activity and expression could then result in transglutaminase bonds in tau monomers, oligomers, and filaments resulting in stable dysfunctional tau monomers and aggregation of stabilized tau filaments. Cross-linked tau may not bind as well to microtubules, resulting in an increase in free tau available for polymer formation. Cross-links may make tau protein resistant to degradation, leading to an increase in tau protein available for filament formation. Alternatively, cross-linking may be the mechanism by which tau assembles into polymers as has been shown to occur in vitro. Lastly, cross-links may stabilize tau polymers in neurofibrillary tangles formed by some other mechanism.

Similarly, transglutaminase-catalyzed cross-linking may lead to the stabilization of huntingtin protein monomers, oligomers, and small polymers. The transglutaminase-modified huntingtin protein is resistant to degradation and could thereby result in increased levels of abnormal huntingtin protein in HD. Transglutaminase-catalyzed
cross-linking may be involved in the pathophysiology of other CAG triplet repeat disorders as well (12, 34, 36). Transglutaminase inhibitors could prevent stabilization of monomers/oligomers and could be tested as possible treatments in these other CAG triplet repeat disorders. Further studies are needed to determine the role(s) of transglutaminase in neurodegenerative diseases. Inhibitors of transglutaminase activity should be explored as potential therapeutic agents in neurodegenerative diseases. Inhibitors of transglutaminase could prevent stabilization of huntingtin in HD and AD as well as in a range of neurodegenerative diseases with protein aggregates as a pathologic feature.

REFERENCES


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