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The multifaceted role of transglutaminase in neurodegeneration: Review article

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Summary. A critical role for transglutaminase [TGase] has been hypothesized in the pathogenesis of the CAG trinucleotide repeat diseases, characterized by proteins with abnormal expansions of a polyglutamine domain. In the last few years the involvement of TGase in neurodegenerative diseases [NDS], including its role in aggregate formation, has been broadened to include Alzheimer's [AD] and Parkinson's Disease [PD]. It is clear that reduction of TGase activity is beneficial for prolonged survival in mouse models of NDS. The pathological progression of these diseases might reflect in part increases of TGase induced aggregates, or changes in other pathways influenced by increases in TGase activity. Neurodegeneration may be influenced by increased TGase activity affecting apoptosis, modulation of GTPase activity and signal transduction. This review will focus on the leading hypotheses in relation to both old and new experimental results.

Keywords: Transglutaminase – Neurodegeneration – Huntington disease – Alzheimer's disease – Parkinson's disease – Cystamine

Abbreviations: TGase, Transglutaminase; HD, Huntington's Disease; AD, Alzheimer's Disease; PD, Parkinson's Disease; NDS, Neurodegenerative Diseases; Gln, Glutamine; SBMA, spinal bulbar muscular atrophy; htt, Huntingtin; NAC, Non-amyloid component; Aβ, amyloid-beta; APP, amyloid precursor protein

Transglutaminase and the post-translational modification of proteins

Clarke and collaborators introduced the term Transglutaminase (TGase) in 1957. They described the enzymatic transamidation of proteins in guinea-pig liver (Clarke et al., 1957). Thereafter, Pisano and colleagues demonstrated that transamidation occurs through cross-linking of proteins through an acyl-transfer reaction between the γ-carboxamide group of peptide-bound glutamine (Gln) and the ε-amino group of peptide-bound lysine, resulting in an $\tilde{\varepsilon}(\gamma$-glutamyl)lysine isopeptide bond (Fig. 1a) (Pisano et al., 1968).

This enzymatic activity required the binding of Ca^{2+}, but at higher concentrations than the physiological range associated with most intracellular processes (Burgoyne and Weiss, 2001). Interestingly, in most NDS, there is evidence for increased intracellular Ca^{2+} concentration, which can be associated with neuronal loss (Lue et al., 1996; Peterson and Goldman, 1986; Ito et al., 1994; Gibson et al., 1996).

A number of competitive inhibitors are available, which block the crosslinking of proteins mediated by TGase. The inhibitor becomes incorporated into the protein substrate and blocks either the acceptor or the donor site. In the case of an amine incorporation, the competitive inhibitor is a primary amine that binds to the acceptor (Fig. 1b), and in the case of acylation the inhibitor is a Gln-containing peptide which blocks the donor (Fig. 1c).

Selkoe provided the first description relating neurodegeneration and TGase activity in 1982. He hypothesized that TGase is involved in NDS, including Parkinson's Disease (PD), Huntington's Disease (HD) and Alzheimer's Disease (AD), because they all might involve aggregate formation in neurons undergoing degeneration. Selkoe and colleagues reported for the first time, that TGase was present and active in brain (Selkoe et al., 1982). Their experiments demonstrated that brain TGase covalently cross-linked brain proteins into insoluble polymers *in vitro*. However, paired helical filaments, one of the types of aggregates present in AD, did not form under the specific *in vitro* conditions. That observation, in part, left open the question of TGase involvement in aggregate

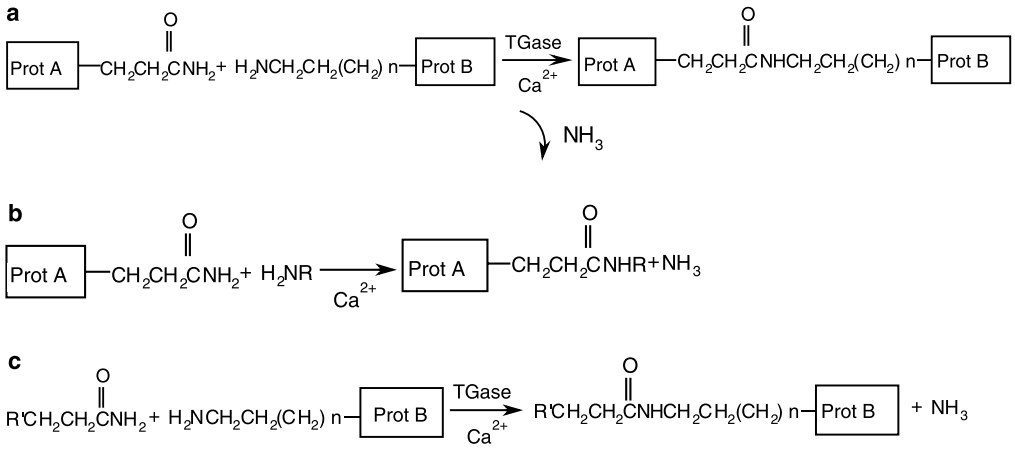

Fig. 1. TGase mediated chemical reactions: **a** Crosslinking of two different proteins where protein A serves as an acceptor while protein B serves as a donor. The product is a covalent bond between protein A and B. Protein A and B are not necessarily two different proteins, resulting in a third possibility where the covalent bond is formed within the same protein. **b** Incorporation of a primary amine or a diamine into protein A results in the future interaction with any other protein containing a potential lysine donor. **c** Acylation of protein B in the presence of a peptide that contains a glutamine residue as an acceptor

formation in NDS, and may have discouraged further research on TGase in NDS for another decade!

After the ensuing decade of inattention to the role of TGase in NDS, a series of landmark discoveries in the early 90's by Fischbeck and colleagues working on bulbar spinal muscular atrophy (SBMA), and Gussella and colleagues working on HD, showed that expanding polyglutamine repeats were at the center of the genetic basis of a group of diseases called the trinucleotide repeat diseases. Expanding stretches of CAG trinucleotide repeats, encoding Gln in different proteins were found to be the basis of the pathology of SBMA, HD and soon thereafter six other NDS (La Spada et al., 1991; Gusella et al., 1983). In 1993 Howard Green hypothesized that TGase cross-linking of polyglutamine stretches in the hungtingtin protein (htt) was critical for the pathogenesis of HD (Green et al., 1993). Since Green's hypothesis, a series of experiments undertaken in humans, animal, and *in vitro* cell culture models have supported this idea. The possible role of TGase in other NDS that are not caused by an expanding CAG repeat encoding polyglutamine, has been rekindled. Interest in the role of TGase in PD and in AD has sparked new studies in these diseases.

Aggregated proteins in NDS serve as substrates for TGase activity

TGase substrates are commonly found in neuronal aggregates in other NDS. Non-amyloid component (NAC), a target for TGase, is a fragment of alpha-synuclein that is present in an aggregated form in Lewy bodies of PD patients (Arima et al., 1998) and in amyloid plaques of AD patients (Wirths et al., 2000). Interestingly, aggregated NAC is more toxic than its monomer (El-Agnaf et al., 1998; Forloni et al., 2000). In addition to NAC, TGase mediated protein-aggregation in NDS, including the domains which participate in this intramolecular process, was shown for amyloid-beta ($A\beta$), the major component of amyloid plaques in AD, (Rasmussen et al., 1994). $A\beta$ oligomerization is catalyzed via TGase (Ikura et al., 1993; Dudek and Johnson, 1994; Ho et al., 1994). Finally, Tau, the major component of the paired helical filaments, forms insoluble and filamentous oligomeric products in the presence of TGase *in vitro* (Dudek and Johnson, 1993; Appelt and Balin, 1997).

Htt was first demonstrated as an excellent glutamyl-donor substrate for TGase *in vitro* (Kahlem et al., 1996; Cooper et al., 1997; Gentile et al., 1998; Karpuj et al., 1999; Karpuj et al., 2002a). In 1999 we demonstrated that TGase could cross-link soluble mutated htt and transform it into an insoluble aggregated conformation, which is a principal feature of HD (Karpuj et al., 1999). In HD patients a truncated version of the mutated htt is present as a nuclear inclusion in neurons (Davies et al., 1997; DiFiglia et al., 1997; Becher et al., 1998). These inclusions are also present in the cytoplasm (Lunkes et al., 2002). *In vitro* experiments demonstrate that more TGase catalyzed aggregates form, when shorter htt constructs, containing 135 or fewer amino acids are used than when the full-length htt constructs are employed. This dependence on the length of the htt construct is particularly evident, when the polyglutamine domain of htt exceeds the pathologic

threshold of polyQ36. In addition, TGase itself was found to be associated with these htt aggregates *in vitro* (Karpuj et al., 1999). Moreover, additional biochemical support for TGase mediated aggregates comes from the laboratory of Professor Howard Green. Green and colleagues showed recently that mutated htt polymers are resistant to denaturation by formic acid, which further suggests the participation of covalent bonds (Iuchi et al., 2003).

TGase activity is elevated in NDS in brain

Besides these *in vitro* experiments, there are *in vivo* results that further indicate a role for TGase in NDS. Immunohistochemistry revealed the co-localization of TGase with amyloid plaques and neurofibrillary tangles in the brains of AD patients (Gilad and Varon, 1985; Miller and Anderton, 1986; Appelt and Balin, 1996; Johnson et al., 1997; Kim et al., 1999; Lesort et al., 1999). In addition to the physical proximity of TGase to aggregated proteins, there is evidence for a link between aggregate formation and TGase activity. For example aggregate formation of alpha-synuclein inclusions in cells is augmented by a calcium ionophore, and prevented by a TGase inhibitor (Junn et al., 2003). Similar findings were reported for htt by Igarashi and coworkers in 1998.

Furthermore, TGase was found to be co-localized with alpha-synuclein in the halo of Lewy bodies, in postmortem brain tissue of PD patients (Junn et al., 2003). TGase activity is elevated in the affected areas of AD brains, compared to controls [reference]. This is in agreement with the fact that there are more $\tilde{\varepsilon}(\gamma$-glutamyl)lysine linkages in insoluble proteins in AD brain tissue. Moreover, elevation of two different TGase isoforms-TGase 1 and 2 expression was evident in AD (Kim et al., 1999). TGase activity was also increased in brains and cerebrospinal fluid of HD patients (Karpuj et al., 1999; Lesort et al., 1999; Jeitner et al., 2001), and TGase is found in htt aggregates *in vivo* (Zainelli et al., 2003). Whether this increased activity of TGase modulates the progress of the disease, or is a result of the disease progression itself is still an enigma.

Inhibition or ablation of TGase activity prolongs survival of NDS in mouse models

Interference with TGase, either by gene-targeted deletion of TGase, or competitive inhibition of its substrate, prolongs the lifespan in various animal models of HD (Karpuj et al., 2002a; Dedeoglu et al., 2002; Mastroberardino et al., 2002). It is important to note that these maneuvers, effectively impairing TGase activity to some degree, were not able to completely reverse HD-like pathology in animal models of HD. It should be noted, however, that for these fatal models of HD, any prolongation in survival is significant. Moreover, the animal models for HD are an extreme model of the disease, with the poly Q domains exceeding those found in juvenile onset HD, thus possibly setting a higher barrier for any successful therapeutic approach. Therefore, clinical trials in humans, using approaches that interfere with TGase activity might have more favorable results, compared to the stringent animal models. Similar studies with PD and AD animal models should be conducted in order to assess the general effect on prolongation of survival due to reduction of TGase activity in NDS involving aggregate formation.

Is prolongation of survival after interference with TGase associated with reduction in aggregate formation?

As for other NDS that are associated with aggregate formation, it remains unclear whether aggregates are involved in HD pathogenesis, or whether they may have a protective function by sequestering aggregated htt (Ordway et al., 1997; Saudou et al., 1998; Yamamoto et al., 2000; Orr and Zoghbi, 2000; Chen et al., 1996). Three different papers address this question in HD animal models (Karpuj et al., 2002b; Dedeoglu et al., 2002; Mastroberardino et al., 2002). While it is clear from all these studies that inhibition of TGase activity prolongs NDS survival, their conclusions are controversial with respect to its effect on aggregate formation. In our own work (Karpuj et al., 2002a, b), no effect on the number or size of aggregates was observed, when cystamine is used to compete for the TGase substrate. In contrast, Dedeoglu and colleagues demonstrate a reduced number of aggregates using the same TGase inhibitor. These contrasting results might be explained by the fact that Deodoglu et al. started the treatment prenatally, via oral feeding of the pregnant mothers, or at 21 days (i.p.), while Karpuj et al. started at 7 weeks of age (i.p.). This might have important implications for the timing of therapeutic trials directed at reducing TGase activity. As in most degenerative conditions, more robust results might be expected when earlier intervention is instituted. The fact that survival can be prolonged even when treatment is started at 7 weeks, a time when disease is manifest, indicates that impairment of TGase activity is a formidable and encouraging strategy.

The study by Mastroberardino et al. (2002) demonstrated that targeted disruption of the TGase gene in HD transgenic mice increased the number of aggregates. The

R6/1 mice used in their model of HD, obtained from Jackson Laboratories, has a longer incubation time for disease, than the R6/2 mice used by Karpuj et al. and Deodoglu et al. In contrast to their studies with cystamine where survival was prolonged by about 20%, deletion of TGase prolonged the life span in 12%. It is possible that aggregate formation may proceed along different pathways in mice where the gene for TGase is deleted. Perutz and we have argued that aggregate formation might be due in part to TGase induced cross-links, as well as to hydrogen bonded beta sheets (Karpuj et al., 1999; Perutz, 1999; Karpuj et al., 2002b). The two processes need not be mutually exclusive.

In summary, while inhibition of TGase activity appears clearly associated with prolonged survival in all mouse models of HD, there is no clear association of this effect with effects on aggregate formation. This suggests that the reduced life-span in these animals is not related to aggregate formation, and that the effect of TGase inhibition is probably mediated through alternative pathways, not involved in aggregate formation.

Alternative mechanisms for prolonged survival by interference with TGase activity

Evidence for alternative pathways that are involved in the prolongation of survival following competition for TGase substrate, was described in our studies with cystamine. Using DNA microarrays, mice treated with cystamine showed elevated transcription of neuro-protective genes, including DnaJ and osteopontin (Karpuj et al., 2002b). Inhibition of TGase activity also alters the level or location of other essential proteins in neurons that serve as a substrate for TGase. For example glyceraldehyde 3-phosphate dehydrogenase and the alpha-ketogluterate dehydrogenase complex are both inactivated by TGase 2 in the presence of glutamine donors (Cooper et al., 1997a; Gentile et al., 1998). Alterations in these metabolites can modulate cerebral energy metabolism (Gibson et al., 2000). Yet, another possibility is that the elevation of TGase activity influences other critical and potentially pathogenic pathways. Such pathways regulated in part by TGase activity involve apoptosis, and signal transduction (Huo et al., 2003; Nakaoka et al., 1994).

Future goals

Interference with TGase prolongs the survival of several NDS raising several interesting points, worthy of future attention:

Several experiments indicate, in cell culture and in *in vivo* models (Junn et al., 2003; Igarashi et al., 1998; Dedeoglu et al., 2002), that TGase inhibitors down-regulate protein aggregation in NDS. It is important to note that these inhibitors might also have an effect on the down-regulation of caspase-another key enzyme involved in NDS (Ientile et al., 2003; Sanchez Mejia and Friedlander, 2001). It would be interesting to use RNAi or other techniques to inhibit the expression of TGase or specific caspases. Down-regulation of each of them separately in neuronal cell cultures or in primary cultures might reveal contrasting effects of each of these two enzymes on neurodegeneration. The confluence of these two enzymatic pathways, TGase and caspase, might become apparent, since caspase cleaves Htt into the truncated isoform that is present in aggregates of HD patients (Kim et al., 2001). These capase cleaved fragments of htt, might serve a better substrate for TGase.

Since neurodegeneration occurs mainly in brain cells and progresses as we age, it would be interesting to compare TGase activity in mouse CNS neurons to neurons in the periphery at various ages. It would be exciting to generate different transgenic mice which express TGase in a tissue specific manner and cross these mice with the various mouse models for NDS. This could shed more light on the direct effect of TGase on NDS *in vivo*. Similarly it would be useful to use conditional knock in and knock out mice to see whether delayed changes in TGase activity might modulate disease. Thus temporal and spatial control of TGase activity in various models of NDS will provide important insights into the role of this critical enzyme in these devastating diseases. It would be interesting as well to test the role of TGase in other trinucleotide repeat diseases where glutamine expansions play a pathogenic role.

As previously mentioned, there are protein aggregates which are associated with a specific NDS, but in each disease these aggregates contain a mixture of various proteins. In HD it is evident that the polyglutamine repeats in the htt protein serve as one of the substrates for TGase. Since TGase crosslinks Lysines and Glutamines, it is still open where the Lysine donor comes from. Might it be essential, to have another protein, as the lysine donor, associated with htt that initiates the cascade of aggregation (an intermolecular cross-linking)? Or is it sufficient to have htt aggregates (cross-linked with intramolecular or intermolecular bonds)? Are other proteins in these aggregates simply 'chaperones' in aggregation process? This would favor the observation that chaperone molecules and ubiquitinated proteins are associated with aggregates in these diseases.

A good way to explore for which proteins are present in the aggregates would be to use labeled TGase inhibitors.

For example if one uses an amine donor peptide with biotin (Fig. 1b R = Biotin) then immunoprecipitation of the crosslinked product with avidin would presumably fish out the putative interacting proteins which might be involved in these aggregates. It would be interesting to perform this experiment in an inducible cell line and add the inhibitors before and after the induction of the aggregates in the presence or absence of TGase, and to compare at the same time the size of aggregates.

It is intriguing that the targeted gene disruption of three major proteins involved in neurodegenerative diseases, htt, alpha-synuclein, and amyloid precursor protein (APP, which is the precursor of Aβ) does not result in death. That might indicate that the loss of these proteins to cellular metabolism, when they aggregate, is *not* causing neurodegeneration. On the other hand these proteins, htt, alpha synuclein and APP, may serve as seeding material to other proteins that are essential to the cell milieu and which might be recruited by TGase into these aggregates. One such protein is the transcriptional corepressor C-terminal binding protein (Kegel et al., 2002). Finding other interacting proteins present in these aggregates might be important. Once the proteins that comprise aggregates are identified, it would be productive to explore whether over and underexpressing this protein might rescue a neuron from degeneration.

The therapeutic potential of TGase modulation must be explored. The ubiquity of TGase catalyzed reactions in physiological processes such as blood coagulation, make the development of specific inhibitors a high priority. But other wide ranging enzyme systems like cycloxygenases, metalloproteases and HMG coA reductase can be inhibited with beneficial results in the clinic.

If TGase is indeed involved directly in the process of neurodegeneration it would be interesting to understand how it contributes to the specificity of each neurodegenerative conditions. For example neurodegeneration in AD, PD and HD involve very different regions of brain with very different clinical pictures. Different isoforms of TGase or specific cofactors of TGase in these selective areas might explain this phenomenon. Understanding the role of TGase in the pathogenesis of neurodegeneration will undoubtedly teach us a great deal about pathogenesis, and may help in the design of therapies for these devastating conditions.

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References

Applet DM, Kopen GC, Boyne LJ, Balin BJ (1996) Localization of transglutaminase in hippocampal neurons; implications for Alzheimer's disease. J Histochem Cytochem 44: 1421–1427

Appelt DM, Balin BJ (1997) The association of tissue transglutaminase with human recombinant tau results in the formation of insoluble filamentous structures. Brain Res 745: 21–31

Arima K, Ueda K, Sunohara N, Hirari S, Izumiyama Y, Tonozuka-Uehara H, Kawai M (1998) Immunoelectron-microscopic demonstration of NACP/alpha-synuclein-epitopes on the filamentous component of Lewy bodies in Parkinson's disease and in dementia with Lewy bodies. Brain Res 808: 93–100

Becher MW, Kotzuk JA, Sharp AH, Davies SW, Bates GP, Price DL, Ross CA (1998) Intranuclear neuronal inclusions in Huntington's disease and dentatorubral and pallidoluysian atrophy: correlation between the density of inclusions and IT15 CAG triplet repeat length. Neurobiol Dis 4: 387–397

Borchelt DR, Wong PC, Sisodia SS, Price DL (1998) Transgenic mouse models of Alzheimer's disease and amyotrophic lateral sclerosis. Brain Pathol 8: 735–757

Burgoyne RD, Weiss JL (2001) The neuronal Ca^{2+} sensor family of Ca^{2+}-binding proteins. Biochem J 353: 1–12

Chen SY, Wright JW, Barnes CD (1996) The neurochemical and behavioral effects of beta-amyloid peptide (25–35). Brain Res 720: 54–60

Clarke DD, Mycek MJ, Neidle A, Waelsch H (1957) The incorporation of amines into proteins. Arch Biochem Biophys 79: 338–354

Cooper AJ, Sheu KR, Burke JR, Onodera O, Strittmatter WJ, Roses AD, Blass JP (1997a) Transglutaminase-catalyzed inactivation of glyceraldehyde 3-phosphate dehydrogenase and alpha-ketoglutarate dehydrogenase complex by polyglutamine domains of pathological length. Proc Natl Acad Sci 94: 12604–12609

Cooper AJ, Sheu KF, Burke JR, Onodera O, Strittmatter WJ, Roses AD, Blass JP (1997b) Polyglutamine domains are substrates of tissue transglutaminase: does transglutaminase play a role in expanded CAG/poly-Q neurodegenerative diseases? J Neurochem 69: 431–434

DiFiglia M, Sapp E, Chase KO, Davies SW, Bates GP, Vonsattel JP, Aronin N (1997) Aggregation of huntingtin in neuronal intranuclear inclusions and dystrophic neurites in brain. Science 277: 1990–1993

Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, Scherzinger E, Wanker EE, Mangiarini L, Bates GP (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 90: 537–548

Dedeoglu A, Kubilus JK, Jeitner TM, Matson SA, Bogdanov M, Kowall NW, Matson WR, Cooper AJ, Ratan RR, Beal MF, Hersch SM, Ferrante RJ (2002) Therapeutic effects of cystamine in a murine model of Huntington's disease. J Neurosci 22: 8942–8950

Dudek SM, Johnson GV (1994) Transglutaminase facilitates the formation of polymers of the beta-amyloid peptide. Brain Res 651: 129–133

El-Agnaf OM, Jakes R, Curran MD, Middleton D, Ingenito R, Bianchi E, Pessi A, Neill D, Wallace A (1998) Aggregates from mutant and wild-type alpha-synuclein proteins and NAC peptide induce apoptotic cell death in human neuroblastoma cells by formation of beta-sheet and amyloid-like filaments. FEBS Lett 440: 71–75

Forloni G, Bertani I, Calells AM, Thaler F, Invernizzi R (2000) Alpha-synuclein and Parkinson's disease: selective neurodegenerative effect of alpha-synuclein fragment on dopaminergic neurons *in vitro* and *in vivo*. Ann Neurol 47: 632–640

Gentile V, Sepe C, Calvani M, Melone MA, Cotrufo R, Cooper AJ, Blass JP, Peluso G (1998) Tissue transglutaminase-catalyzed formation of

high-molecular-weight aggregates *in vitro* is favored with long polyglutamine domains: a possible mechanism contributing to CAG-triplet diseases. Arch Biochem Biophys 352: 314–321

Gibson GE, Zhang H, Toral-Barza L, Szolosi S, Tofel-Grehl B (1996) Calcium stores in cultured fibroblasts and their changes with Alzheimer's disease. Biochim Biophys Acta 1316: 71–77

Gibson GE, Park LC, Sheu KF, Blass JP, Calingasan NY (2000) The alpha-ketoglutarate dehydrogenase complex in neurodegeneration. Neurochem Int 36: 97–112

Gilad GM, Varon LE (1985) Transglutaminase activity in ret brain: characterization, distribution, and changes with age. J Neurochem 45: 1522–1526

Green H (1993) Human genetic diseases due to codon reiteration: relationship to an evolutionary mechanism. Cell 74: 955–956

Guenette ST, Tanzi RE (1999) Progress towards valid transgenic mouse models for Alzheimer's disease. Neurobiol Aging 20: 201–211

Gusella JF, Wexler NS, Conneally PM, Naylor SL, Anderson MA, Tanzi RE, Watkins PC, Ottina K, Wallace MR, Sakaguchi AY, et al (1983) A polymorphic DNA marker genetically linked to Huntington's disease. Nature 306: 234–238

Ho GJ, Gregory EJ, Smirnova IV, Zoubine MN, Festoff BW (1994) Cross-linking of beta-amyloid protein precursor catalyzed by tissue transglutaminase. FEBS Lett 349: 151–154

Huo J, Metz SA, Li G (2003) Role of tissue transglutaminase in GTP depletion-induced apoptosis of insulin-secreting (HIT-T15) cells. Biochem Pharmacol 66: 213–223

Ientile R, Campisi A, Raciti G, Caccamo D, Curro M, Cannavo G, Li Volti G, Macaione S, Vanella A (2003) Cystamine inhibits transglutaminase and caspase-3 cleavage in glutamate-exposed astroglial cells. J Neurosci Res 74: 52–59

Igarashi S, Koide R, Shimohata T, Yamada M, Hayashi Y, Takano H, Date H, Oyake M, Sato T, Sato A, Egawa S, Ikeuchi T, Tanaka H, Nakano R, Tanaka K, Hozumi I, Inuzuka T, Takahashi H, Tsuji S (1998) Suppression of aggregate formation and apoptosis by transglutaminase inhibitors in cells expressing truncated DRPLA protein with an expanded polyglutamine stretch. Nat Genet 18: 111–117

Ikura K, Takahata K, Sasaki R (1993) Cross-linking of a synthetic partial-length (1–28) peptide of the Alzheimer beta/A4 amyloid protein by transglutaminase. FEBS Lett 326: 109–111

Ito E, Oka K, Etcheberrigaray R, Nelson TJ, McPhie DL, Tofel-Grehl B, Gibson GE, Alkon DL (1994) Internal Ca^{2+} mobilization is altered in fibroblasts from patients with Alzheimer disease. Proc Natl Acad Sci USA 91: 534–538

Iuchi S, Hoffner G, Verbeke P, Djian P, Green H (2003) Oligomeric and polymeric aggregates formed by proteins containing expanded polyglutamine. Proc Natl Acad Sci USA 100: 2409–2414

Jeitner TM, Bogdanov MB, Matson WR, Daikhin Y, Yudkoff M, Folk JE, Steinman L, Browne SE, Beal MF, Blass JP, Cooper AJ (2001) N(epsilon)-(gamma-L-glutamyl)-L-lysine (GGEL) is increased in cerebrospinal fluid of patients with Huntington's disease. J Neurochem 79: 1109–1112

Johnson GV, Cox TM, Lockhart JP, Zinnerman MD, Miller ML, Powers RE (1997) Transglutaminase activity is increased in Alzheimer's disease in brain. Brain Res 751: 323–329

Junn E, Ronchetti RD, Quezado MM, Kim SY, Mouradian MM (2003) Tissue transglutaminase-induced aggregation of alpha-synuclein: implications for Lewy body formation in Parkinson's disease and dementia with Lewy bodies. Proc Natl Acad Sci USA 100: 2047–2052

Kahlem P, Terre C, Green H, Djian P (1996) Peptides containing glutamine repeats as substrates for transglutaminase-catalyzed cross-linking: relevance to diseases of the nervous system. Proc Natl Acad Sci USA 93: 14580–14585

Karpuj MV, Garren H, Slunt H, Price DL, Gusella J, Becher MW, Steinman L (1999) Transglutaminase aggregates huntingtin into non-amyloidogenic polymers, and its enzymatic activity increases in Huntington's disease brain nuclei. Proc Natl Acad Sci USA 96: 7388–7393

Karpuj MV, Becher MW, Springer JE, Chabas D, Youssef S, Pedotti R, Mitchell D, Steinman L (2002a) Prolonged survival and decreased abnormal movements in transgenic model of Huntington disease, with administration of the transglutaminase inhibitor cystamine. Nat Med 8: 143–149

Karpuj MV, Becher MW, Steinman L (2002b) Evidence for a role for transglutaminase in Huntington's disease and the potential therapeutic implications. Neurochem Int 40: 31–36

Kegel KB, Meloni AR, Yi Y, Kim YJ, Doyle E, Cuiffo BG, Sapp E, Wang Y, Qin ZH, Chen JD, Nevins JR, Aronin N, DiFiglia M (2002) Huntingtin is present in the nucleus, interacts with the transcriptional corepressor C-terminal binding protein, and represses transcription. J Biol Chem 277: 7466–7476

Kim SY, Grant P, Lee JH, Pant HC, Steinert PM (1999) Differential expression of multiple transglutaminases in human brain. Increased expression and cross-linking bt transglutaminase 1 and 2 in AD. J Biol Chem 274: 30715–30721

Kim YJ, Yi Y, Sapp E, Wang Y, Cuiffo B, Kegel KB, Qin ZH, Aronin N, DiFiglia M (2001) Caspase 3-cleaved N-terminal fragments of wild-type and mutant huntingtin are present in normal and Huntington's disease brains, associate with membranes, and undergo calpain-dependent proteolysis. Proc Natl Acad Sci USA 98: 12784–12789

La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. Nature 352: 77–79

Lesort M, Chun W, Johnson GVW, Ferrante RJ (1999) Tissue transglutaminase in increased in Huntington's disease brain. J Neurochem 73: 2018–2027

Lunkes A, Lindenberg KS, Ben-Haiem L, Weber C, Devys D, Landwehrmeyer GB, Mandel JL, Trottier Y (2002) Proteases acting on mutant huntingtin generate cleaved products that differentially build up cytoplasmic and nuclear inclusions. Mol Cell 10: 259–269

Lue LF, Brachova L, Civin WH, Rogers J (1996) Inflammation A beta deposition, and neurofibrillary tangle formation as correlates of Alzheimer's disease neurodegeneration. J Neuropathol Exp Neurol 55: 1083–1088

Miller CC, Anderton BH (1986) Transglutaminase and the neuronal cytoskeleton in Alzheimer's disease. J Neurochem 46: 1912–1922

Mastroberardino PG, Iannicola C, Nardacci R, Bernassola F, De Laurenzi V, Melino G, Moreno S, Pavone F, Oliverio S, Fesus L, Piacentini M (2002) 'Tissue' transglutaminase ablation reduces neuronal death and prolongs survival in a mouse model of Huntington's disease. Cell Death Differ 9: 873–880

Nakaoka H, Perez DM, Baek KJ, Das T, Husain A, Misono K, Im MJ, Graham RM (1994) Gh: a GTP-binding protein with transglutaminase activity and receptor signaling function. Science 264: 1593–1596

Ordway JM, Tallaksen-Greene S, Gutekunst CA, Bernstein EM, Cearley JA, Wiener HW, Dure IV LS, Lindsey R, Hersch SM, Jope RS, Albin RL, Detloff PJ (1997) Ectopically Expressed CAG Repeats Cause Intranuclear Inclusions and a Progressive Late Onset Neurological Phenotype in the mouse. Cell 91: 753–763

Orr H, Zoghbi H (2000) Reversing neurodegeneration: a promise unfolds. Cell 101: 1–4

Perutz MF (1999) Glutamine repeats and neurodegenerative diseases: molecular aspects. Trends Biochem Sci 24: 58–63

Peterson C, Goldman JE (1986) Alterations in calcium content and biochemical processes in cultured skin fibroblasts from aged and Alzheimer donors. Proc Natl Acad Sci 83: 2758–2762

Pisano JJ, Finlayson JS, Peyton MP (1968) Cross-link in fibrin polymerized by factor 13: epsilon-(gamma-glutamyl)lysine. Science 160: 892–893

Rasmussen LK, Sorensen ES, Petersen TE, Gliemann J, Jensen PH (1994) Identification of glutamine and lysine residues in Alzheimer amyloid

beta A4 peptide responsible for transglutaminase-catalysed homopolymerization and cross-linking to alpha 2 M receptor. FEBS Lett 338: 161–166

Sanchez Mejia RO, Friedlander RM (2001) Caspases in Huntington's disease. Neuroscientist 7: 480–489

Sathasivam K, Hobbs C, Mangiarini L, Mahal A, Turmaine M, Doherty P, Davies SW, Bates GP (1999) Transgenic models of Huntington's disease. Philos Trans R Soc Lond B Biol Sci 354: 963–969

Saudou P, Finkbeiner S, Devys D, Greenberg M (1998) Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell 95: 55–66

Selkoe DJ, Abraham C, Ihara Y (1982) Brain transglutaminase: *in vitro* crosslinking of human neurofilament proteins into insoluble polymers. PNAS 79: 6070–6074

Su JH, Anderson AJ, Cummings BJ, Cotman CW (1994) Immunohistochemical evidence for apoptosis in Alzheimer's disease. Neuroreport 5: 2529–2533

Turmaine M, Raza A, Mahal A, Mangiarini L, Bates GP, Davies SW (2000) Nonapoptotic neurodegeneration in a transgenic mouse model of Huntington's disease. Proc Natl Acad Sci 97: 8093–8097

Wirths O, Weickert S, Majtenyi K, Havas L, Kahle PJ, Okochi M, Haass C, Multhaup G, Beyreuther K, Bayer TA (2000) Lewy body variant of Alzheimer's disease: alpha-synuclein in dystrophic neuritis of A beta plaques. Neororeport 11: 3737–3741

Yamamoto A, Lucas JJ, Hen R (2000) Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. Cell 101: 57–66

Zainelli GM, Ross CA, Troncoso JC, Muma NA (2003) Transglutaminase cross-links in intranuclear inclusions in Huntington disease. J Neuropathol Exp Neurol 62: 14–24

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