World Journal of Biological Chemistry

Online Submissions: http://www.wjgnet.com/1949-8454office
wjbc@wjgnet.com
doi:10.4331/wjbc.v1.i5.181

World J Biol Chem 2010 May 26; 1(5): 181-187
ISSN 1949-8454 (online)

REVIEW

Physio-pathological roles of transglutaminase-catalyzed reactions

Mariangela Ricotta, Maura Iannuzzi, Giulia De Vivo, Vittorio Gentile

Mariangela Ricotta, Maura Iannuzzi, Giulia De Vivo, Vittorio Gentile, Department of Biochemistry and Biophysics, Medical School, Second University of Naples, via Costantinopoli 16, 80138 Napoli, Italy

Author contributions: Ricotta M and Iannuzzi M performed part of experiments; De Vivo G designed and performed part of experiments; Gentile V co-ordinated and performed experiments and wrote the paper.

Correspondence to: Vittorio Gentile, MD, PhD, Neurologist, Aggregate Professor of Biochemistry, Department of Biochemistry and Biophysics, Medical School, Second University of Naples, via Costantinopoli 16, 80138 Napoli, Italy. vittorio.gentile@unina2.it

Telephone: +39-81-5665870 Fax: +39-81-5665863

Received: April 24, 2010 Revised: May 4, 2010
Accepted: May 14, 2010
Published online: May 26, 2010

Abstract

Transglutaminases (TGs) are a large family of related and ubiquitous enzymes that catalyze post-translational modifications of proteins. The main activity of these enzymes is the cross-linking of a glutaminyl residue of a protein/peptide substrate to a lysyl residue of a protein/peptide co-substrate. In addition to lysyl residues, other second nucleophilic co-substrates may include monoamines or polyamines (to form mono- or bi-substituted /crosslinked adducts) or -OH groups (to form ester linkages). In the absence of co-substrates, the nucleophile may be water, resulting in the net deamidation of the glutaminyl residue. The TG enzymes are also capable of catalyzing other reactions important for cell viability. The distribution and the physiological roles of TG enzymes have been widely studied in numerous cell types and tissues and their roles in several diseases have begun to be identified. "Tissue" TG (TG2), a member of the TG family of enzymes, has definitely been shown to be involved in the molecular mechanisms responsible for a very widespread human pathology: i.e. celiac disease (CD). TG activity has also been hypothesized to be directly involved in the pathogenetic mechanisms responsible for several other human diseases, including neurodegenerative diseases, which are often associated with CD. Neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, supranuclear palsy, Huntington's disease and other recently identified polyglutamine diseases, are characterized, in part, by aberrant cerebral TG activity and by increased cross-linked proteins in affected brains. In this review, we discuss the physio-pathological role of TG-catalyzed reactions, with particular interest in the molecular mechanisms that could involve these enzymes in the physio-pathological processes responsible for human neurodegenerative diseases.

Key words: Transglutaminases; Post-translational-modifications of proteins; Celiac disease; Neurodegenerative diseases; Transglutaminase inhibitors

Peer reviewer: Wayne Grant Carter, PhD, School of Biomedical Sciences, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH, United Kingdom

Ricotta M, Iannuzzi M, De Vivo G, Gentile V. Physio-pathological roles of transglutaminase-catalyzed reactions. *World J Biol Chem* 2010; 1(5): 181-187 Available from: URL: http://www.wjgnet.com/1949-8454/full/v1/i5/181.htm DOI: http://dx.doi.org/10.4331/wjbc.v1.i5.181

BIOCHEMISTRY OF THE TRANSGLUTAMINASES

Transglutaminases (TGs, E.C. 2.3.2.13) catalyze irreversible post-translational modifications of proteins. Examples of TG-catalyzed reactions include: (1) acyl transfer between the γ-carboxamide group of a protein/polypeptide glutaminyl residue and the ε-amino group of

a protein/polypeptide lysyl residue; (2) attachment of a polyamine to the γ-carboxamide of a glutaminyl residue; and (3) deamidation of the γ-carboxamide group of a protein/polypeptide glutaminyl residue (Figure 1)[1,2]. The reactions catalyzed by TGs occur as a two-step mechanism (Figure 2). The transamidating activity of TGs is activated by the binding of Ca^{2+}, which exposes an active-site cysteine residue. This cysteine residue reacts with the γ-carboxamide group of an incoming glutaminyl residue of a protein/peptide substrate to yield a thioacyl-enzyme intermediate and ammonia (Figure 2, Step 1). The thioacyl-enzyme intermediate then reacts with a nucleophilic primary amine substrate, resulting in the covalent attachment of the amine-containing donor to the substrate glutaminyl acceptor and regeneration of the cysteinyl residue at the active site, (Figure 2, Step 2). If the primary amine is donated by the ε-amino group of a lysyl residue in a protein/polypeptide, a N^{ε}-(γ-L-glutamyl)-L-lysine (GGEL) isopeptide bond is formed, (Figure 1A). On the other hand, if a polyamine or another primary amine (e.g. histamine) acts as the amine donor, a γ-glutamylpolyamine (or γ-glutamylamine) residue is formed (Figure 1B). It is also possible for a polyamine to act as a N,N-bis-(γ-L-glutamyl) polyamine bridge between two glutaminyl acceptor residues either on the same protein/polypeptide or between two proteins/polypeptides[3]. If there is no primary amine present, water may act as the attacking nucleophile, resulting in the deamidation of glutaminyl residues to glutamyl residues (Figure 1C). It is worth noting that two of these reactions, in particular, the deamidation of peptides obtained from the digestion of the gliadin, a protein present in wheat, and the GGEL isopeptide formation between these peptides and "tissue" TG (TG2 or tTG), have been recently shown to cause the formation of new antigenic epitopes, which are responsible for immunological reactions during celiac disease (CD), one of the most common human autoimmune diseases[4,5]. The reactions catalyzed by TGs occur with little change in free energy and hence should theoretically be reversible. However, under physiological conditions the cross linking reactions catalyzed by TGs are usually irreversible. This irreversibility partly results from the metabolic removal of ammonia from the system and from thermodynamic considerations resulting from altered protein conformation. Some scientific reports suggest that TGs may be able to catalyze the hydrolysis of GGEL cross-links isopeptide bonds in some soluble cross-linked proteins. Furthermore, it is likely that TGs can catalyze the exchange of polyamines into proteins[2]. In some TGs, other catalytic activities, such as the ability to hydrolyze GTP (or ATP) into GDP (or ADP) and inorganic phosphate, a protein disulfide isomerase activity, a serine/threonine kinase activity and an esterification activity, are often present[6-9].

TGS ARE MULTIFUNCTIONAL ENZYMES

Numerous studies have indicated that some TGs are multifunctional proteins with distinct and regulated enzymatic activities. In fact, under physiological conditions, the transamidation activity of TGs is latent[10], while other activities, recently identified, could be present. For example, in some pathophysiological states, when the concentration of Ca^{2+} increases, the crosslinking activity of TGs may contribute to important biological processes. As previously described, one of the most intriguing properties of some TGs, such as TG2, is the ability to bind and hydrolyze GTP and, furthermore, to bind to GTP and Ca^{2+}. GTP and Ca^{2+} regulate its enzymatic activities, including protein cross-linking, in a reciprocal manner: the binding of Ca^{2+} inhibits GTP-binding and GTP-binding inhibits the TG cross-linking activity of TG2[6]. Interestingly, TG2 shows no sequence homology with heterotrimeric or low-molecular-weight G-proteins, but there is evidence that TG2 (TG2/Ghα) is involved in signal transduction and, therefore, TG2/Ghα should also be classified as a large molecular weight G-protein. Other studies, along with this study, showed that TG2/Ghα can mediate the activation of phospholipase C (PLC) by the α_{1b}-adrenergic receptor[11] and can modulate adenylyl cyclase activity[12]. TG2/Ghα can also mediate the activation of the δ1 isoform of PLC and of maxi-K channels[13]. Interestingly, the signaling function of TG2/Ghα is preserved even with the mutagenic inactivation of its crosslinking activity by the mutation of the active site cysteine residue[14]. However, evidence for a pathophysiological role of the TGs in cell signaling, in disulfide isomerase activity and in other biological functions is still lacking.

Table 1 TG enzymes and their biological functions when known

TG	Physiological role	Gene map location	Ref.
Factor XIIIa	Blood clotting	6p24-25	[15]
TG1 (Keratinocyte TG, kTG)	Skin differentiation	14q11.2	[16]
TG2 (Tissue TG, tTG, cTG)	Apoptosis, cell adhesion, signal transduction	20q11-12	[17]
TG3 (Epidermal TG, eTG)	Hair follicle differentiation	20p11.2	[18]
TG4 (Prostate TG, pTG)	Suppression of sperm immunogenicity	3q21-2	[19]
TG5 (TG X)	Epidermal differentiation	15q15.2	[20]
TG6 (TG Y)	Unknown function	20p13	[20]
TG7 (TG Z)	Unknown function	15q15.2	[20]

TG: Transglutaminases.

MOLECULAR BIOLOGY OF THE TRANGLUTAMINASES

To date, at least eight different TGs, distributed in the human body, have been identified (Table 1). Complex mechanisms regulating the gene expression of TGs, both at transcriptional and translational levels, determine a complex but precise distribution of these enzymes in a cell and/or a tissue[21]. Such complex gene expression reflects the physiological roles that these enzymes play in both the intracellular and extracellular compartments.

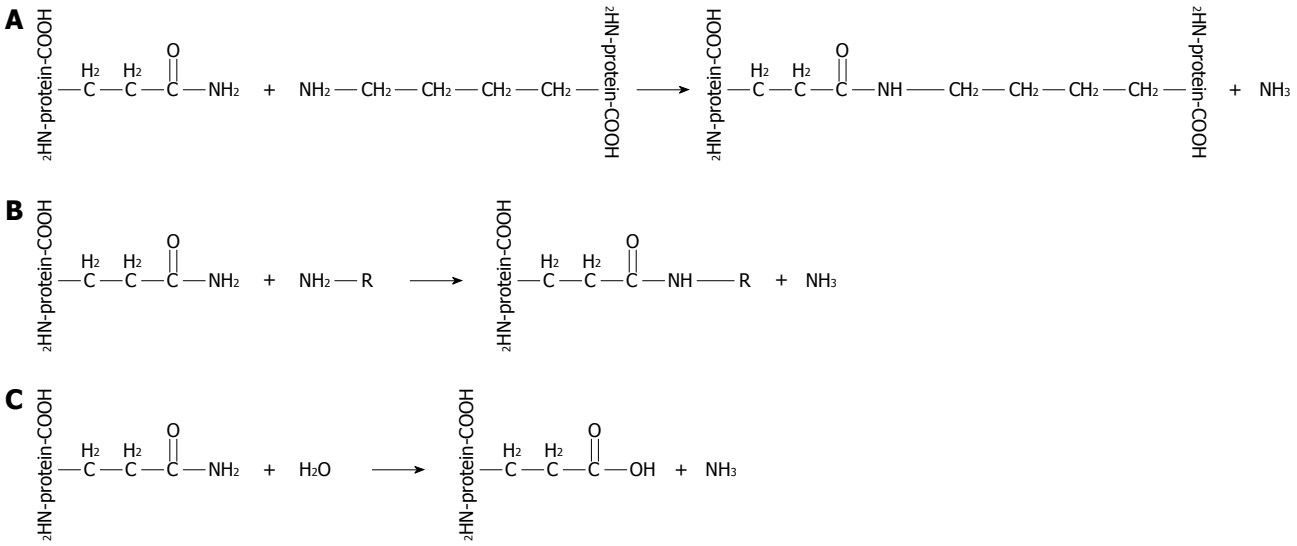

Figure 1 Transglutaminase (TG)-catalyzed reactions. R: Monoamines, polyamines. Examples of TG-catalyzed reactions: A: Acyl transfer between the γ-carboxamide group of a protein/polypeptide glutaminyl residue and the ε-amino group of a protein/polypeptide lysyl residue; B: Attachment of a polyamine to the carboxamide group of a glutaminyl residue; C: Deamidation of the γ-carboxamide group of a protein/polypeptide glutaminyl residue.

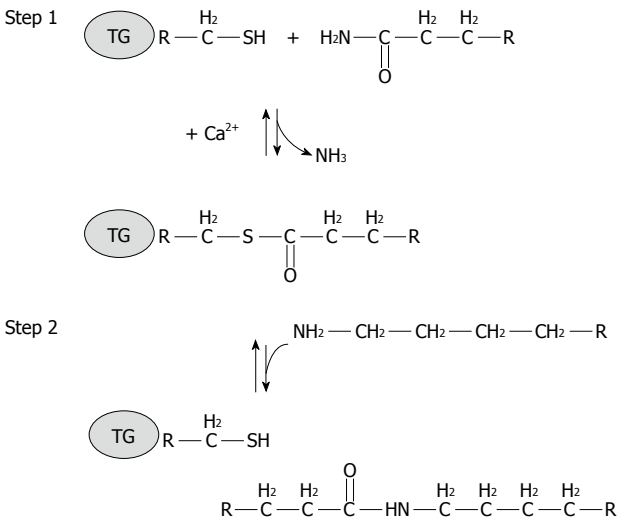

Figure 2 Schematic representation of a two step transglutaminase reaction. Step 1: In the presence of Ca^{2+}, the active-site cysteine residue reacts with the γ-carboxamide group of an incoming glutaminyl residue of a protein/peptide substrate to yield a thioacyl-enzyme intermediate and ammonia; Step 2: The thioacyl-enzyme intermediate reacts with a nucleophilic primary amine substrate, resulting in the covalent attachment of the amine-containing donor to the substrate glutaminyl acceptor and regeneration of the cysteinyl residue at the active site. If the primary amine is donated by the ε-amino group of a lysyl residue in a protein/polypeptide, a N^{ε}-(γ-L-glutamyl)-L-lysine (GGEL) isopeptide bond is formed.

In the nervous system, for example, several forms of TGs are simultaneously expressed[20,22,23]. Moreover, several alternative splice variants of TGs, mostly in the 3'-end region, have been identified. Interestingly, some of them are differently expressed in human pathologies, such as Alzheimer's disease (AD)[24]. On the basis of their ubiquitous expression and their biological roles, we may speculate that the absence of these enzymes would be lethal. However, this does not always seem to be the case, since, for example, null mutants of TG2 are usually phenotypically normal at birth[25]. This result may be explained by multiple expressions of other TG genes that could be substituting for the missing isoform.

Bioinformatic studies have shown that the primary structures of human TGs share some identities in only a few regions, such as the active site and the calcium binding regions. However, high sequence conservation and, therefore, a high degree of preservation of residue secondary structure among TG2, TG3 and FXIIIa indicate that these TGs all share four-domain tertiary structures, which could be similar to those of other TGs[26].

TGS AND NEURODEGENERATIVE DISEASES

An ever-growing number of scientific reports suggest that TG activity is involved in the pathogenesis of neurodegenerative diseases. To date, however, mainly indirect evidence has been obtained about the involvement of these enzymes in the pathophysiology of these neurological diseases. Protein aggregates in affected brain regions are histopathological hallmarks of many neurodegenerative diseases[27]. More than 20 years ago, Selkoe *et al*[28] suggested that TG activity might contribute to the formation of protein aggregates in AD brains. In support of this hypothesis, tau protein has been shown to be an excellent *in vitro* substrate of TGs[29] and GGEL crosslinks have been found in the neurofibrillary tangles and paired helical filaments of AD brains[30]. Interestingly, a recent study showed the presence of bis γ-glutamyl putrescine in human cerebrospinal fluid (CSF), which was increased in Huntington's disease (HD) CSF[31]. This is important evidence that protein/peptides crosslinking by polyamines does indeed occur in the brain, and that this is increased in HD brains. More recently, TG activity has been shown to induce amyloid β-protein oligomerization and aggregation at physiologic levels[32]. By these molecu-

Table 2 List of polyglutamine (CAG-expansion) diseases

Disease	Sites of neuropathology	CAG triplet number		Gene product (Intracellular localization of protein deposits)	Ref.
		Normal	Disease		
Corea major or HD	Striatum (medium spiny neurons) and cortex in late stage	6-35	36-121	Huntingtin (n, c)	[44]
SCA1	Cerebellar cortex (Purkinje cells), dentate nucleus and brain stem	6-39	40-81	Ataxin-1 (n, c)	[45]
SCA2	Cerebellum, pontine nuclei, substantia nigra	15-29	35-64	Ataxin-2 (c)	[46]
SCA3 or MJD	Substantia nigra, globus pallidus, pontine nucleus, cerebellar cortex	13-42	61-84	Ataxin-3 (c)	[47]
SCA6	Cerebellar and mild brainstem atrophy	4-18	21-30	Calcium channel Subunit (a1A) (m)	[48]
SCA7	Photoreceptor and bipolar cells, cerebellar cortex, brainstem	7-17	37-130	Ataxin-7 (n)	[49]
SCA12	Cortical, cerebellar atrophy	7-32	41-78	Brain specific regulatory subunit of protein phosphatase PP2A (?)	[50]
SCA17	Gliosis and neuronal loss in the Purkinje cell layer	29-42	46-63	TATA-binding protein (TBP) (n)	[51]
SBMA or Kennedy disease	Motor neurons (anterior horn cells, bulbar neurons) and dorsal root ganglia	11-34	40-62	Androgen receptor (n, c)	[52]
DRPLA	Globus pallidus, dentato-rubral and subthalamic nucleus	7-35	49-88	Atrophin (n, c)	[53]

HD: Huntington's disease; SCA: Spinocerebellar ataxia; MJD: Machado-Joseph disease; SBMA: Spinobulbar muscular atrophy; DRPLA: Dentatorubral-pallidoluysian atrophy; c: Cytosolic; m: Transmembrane; n: Nuclear.

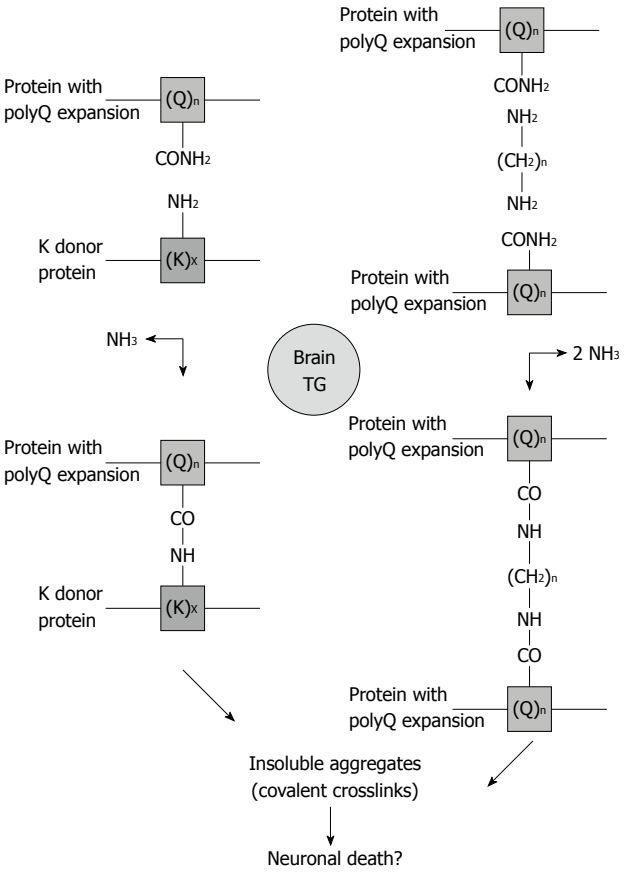

Figure 3 Possible mechanisms responsible for protein aggregate formation catalyzed by transglutaminase.

lar mechanisms, TGs could contribute to AD symptoms and progression[32]. Moreover, there is evidence that TGs also contribute to the formation of proteinaceous deposits in Parkinson's disease (PD)[33,34], in supranuclear palsy[35,36] and in HD, a neurodegenerative disease caused by a CAG expansion in the affected gene[37]. For example, expanded polyglutamine domains have been reported to be substrates of TG2[38-40] and therefore aberrant TG activity could contribute to CAG-expansion diseases. However, although all these studies suggest the possible involvement of TGs in the formation of deposits of protein aggregates in neurodegenerative diseases, they do not indicate whether aberrant TG activity per se directly determines disease progression. For example, several experimental findings reported that TG2 activity *in vitro* leads to the formation of soluble aggregates of α-synuclein[41] or polyQ proteins[42,43]. To date, as previously reported, at least ten human CAG-expansion diseases have been described (Table 2) and, in at least eight of them, their neuropathology is caused by the expansion in the number of residues in the polyglutamine domain to a value beyond 35-40. Remarkably, the mutated proteins have no obvious similarities except for the expanded polyglutamine domain. Most of the mutated proteins are widely expressed both within the brain and elsewhere in the body. A major challenge then is to understand why the brain is primarily affected and why different regions within the brain are affected in the different CAG-expansion diseases; i.e. what accounts for the neurotoxic gain of function for each protein and for a selective vulnerability of each cell type. Possibly, the selective vulnerability[54] may be explained in part by the susceptibility of the expanded polyglutamine domains in the various CAG-expansion diseases to act as co-substrates for a brain TG, as shown in Figure 3. To strengthen the possible central role of the TGs in neurodegenerative diseases, a study by Hadjivassiliou *et al*[55] showed that anti-TG2 IgA antibodies are present in the gut and brain of patients with gluten ataxia, a non-genetic sporadic cerebellar ataxia, but not in ataxia control patients. Recently, anti-TG2, -TG3 and -TG6 antibodies have been found in sera from CD patients, suggesting a possible involvement also of other TGs in the pathogenesis of dermatitis herpetiformis and gluten ataxia, two frequent extraintestinal manifestations of gluten sensitivity[56,57]. Therefore, these studies suggest that the involvement of brain TGs could represent a common denominator in several neurodegenerative

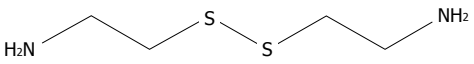

Figure 4 Chemical structure of cystamine.

diseases, which can lead to the determination of pathophysiological consequences through different molecular mechanisms (e.g. biochemical or immunological).

TGS AS POTENTIAL THERAPEUTIC TARGETS OF NEURODEGENERATIVE DISEASES

Since there have been no long-term effective treatments for these human neurodegenerative diseases until now, the possibility that selective TG inhibitors may be of clinical benefit has been seriously considered. In this respect, some encouraging results have been obtained with TG inhibitors in preliminary studies with different biological models of CAG-expansion diseases. For example, cystamine (Figure 4) is a potent *in vitro* inhibitor of enzymes that require an unmodified cysteine at the active site[58]. In as much as TGs contain a crucial active-site cysteine, cystamine has the potential to inhibit these enzymes by disulfide interchange reactions. A disulfide interchange reaction results in the formation of cysteamine and a cysteamine-cysteine mixed disulfide residue at the active site. Recent studies have shown that cystamine decreases the number of protein inclusions in transfected cells expressing the atrophin protein containing a pathological-length polyglutamine domain[59]. In other studies, cystamine administration to HD-transgenic mice resulted in an increase in life expectancy and amelioration of neurological symptoms[60,61]. Neuronal inclusions were decreased in one of these studies[60]. Although all these scientific reports seem to support the hypothesis of a direct role of TG activity in the pathogenesis of polyglutamine diseases, cystamine is also found to act in the HD-transgenic mice by mechanisms other than the inhibition of TGs, such as the inhibition of caspases[62], suggesting that this compound can have an additive effect in the therapy of HD. The pharmacodynamics and the pharmacokinetics of cystamine, therefore, should be carefully investigated in order to confirm the same effectiveness in patients with HD and possibly in patients with other neurodegenerative diseases. Another critical problem in the use of TG inhibitors in treating neurological diseases relates to the fact that, as previously reported, the human brain contains at least four TGs, including TG1, TG2, TG3[17] and possibly TG6[63], and a strong non-selective inhibitor of TGs might also inhibit plasma Factor XIIIa, causing a bleeding disorder. Therefore, from a number of standpoints it would seem that a selective inhibitor that discriminates among TGs would be preferable to an indiscriminate TG inhibitor. Finally, most of the TG activity in mouse brain, at least as assessed by an assay that measures the incorporation of radioactive putrescine (amine donor) into N,N-dimethyl casein (amine acceptor) seems to be due to TG2[64]. However, no conclusive data has been obtained about the involvement of this TG in the development of symptoms in HD-transgenic mice in TG2 gene knock-out experiments[65].

CONCLUSION

In conclusion, although many scientific reports have implicated aberrant TG activity in neurodegenerative diseases, today we are still looking for data that could definitely confirm the direct involvement of TGs in the pathogenetic mechanisms responsible for these diseases. The use of inhibitors of TGs could then be useful in experimental approaches. To minimize the possible side effects, however, selective inhibitors of the TGs should be considered. Progress in this area of research may be achieved in the near future through pharmaco-genetic techniques.

REFERENCES

1 **Folk JE**. Mechanism and basis for specificity of transglutaminase-catalyzed epsilon-(gamma-glutamyl) lysine bond formation. *Adv Enzymol Relat Areas Mol Biol* 1983; **54**: 1-56
2 **Lorand L**, Conrad SM. Transglutaminases. *Mol Cell Biochem* 1984; **58**: 9-35
3 **Piacentini M**, Martinet N, Beninati S, Folk JE. Free and protein-conjugated polyamines in mouse epidermal cells. Effect of high calcium and retinoic acid. *J Biol Chem* 1988; **263**: 3790-3794
4 **Kim CY**, Quarsten H, Bergseng E, Khosla C, Sollid LM. Structural basis for HLA-DQ2-mediated presentation of gluten epitopes in celiac disease. *Proc Natl Acad Sci USA* 2004; **101**: 4175-4179
5 **Fleckenstein B**, Qiao SW, Larsen MR, Jung G, Roepstorff P, Sollid LM. Molecular characterization of covalent complexes between tissue transglutaminase and gliadin peptides. *J Biol Chem* 2004; **279**: 17607-17616
6 **Achyuthan KE**, Greenberg CS. Identification of a guanosine triphosphate-binding site on guinea pig liver transglutaminase. Role of GTP and calcium ions in modulating activity. *J Biol Chem* 1987; **262**: 1901-1906
7 **Hasegawa G**, Suwa M, Ichikawa Y, Ohtsuka T, Kumagai S, Kikuchi M, Sato Y, Saito Y. A novel function of tissue-type transglutaminase: protein disulphide isomerase. *Biochem J* 2003; **373**: 793-803
8 **Lahav J**, Karniel E, Bagoly Z, Sheptovitsky V, Dardik R, Inbal A. Coagulation factor XIII serves as protein disulfide isomerase. *Thromb Haemost* 2009; **101**: 840-844
9 **Iismaa SE**, Mearns BM, Lorand L, Graham RM. Transglutaminases and disease: lessons from genetically engineered mouse models and inherited disorders. *Physiol Rev* 2009; **89**: 991-1023
10 **Smethurst PA**, Griffin M. Measurement of tissue transglutaminase activity in a permeabilized cell system: its regulation by Ca2+ and nucleotides. *Biochem J* 1996; **313** (Pt 3): 803-808
11 **Nakaoka H**, Perez DM, Baek KJ, Das T, Husain A, Misono K, Im MJ, Graham RM. Gh: a GTP-binding protein with transglutaminase activity and receptor signaling function. *Science* 1994; **264**: 1593-1596
12 **Gentile V**, Porta R, Chiosi E, Spina A, Valente F, Pezone R, Davies PJ, Alaadik A, Illiano G. tTGase/G alpha h protein expression inhibits adenylate cyclase activity in Balb-C 3T3 fibroblasts membranes. *Biochim Biophys Acta* 1997; **1357**: 115-122
13 **Nanda N**, Iismaa SE, Owens WA, Husain A, Mackay F, Graham RM. Targeted inactivation of Gh/tissue transglutaminase II. *J Biol Chem* 2001; **276**: 20673-20678

14 **Mian S**, el Alaoui S, Lawry J, Gentile V, Davies PJ, Griffin M. The importance of the GTP-binding protein tissue transglutaminase in the regulation of cell cycle progression. *FEBS Lett* 1995; **370**: 27-31
15 **Olaisen B**, Gedde-Dahl T Jr, Teisberg P, Thorsby E, Siverts A, Jonassen R, Wilhelmy MC. A structural locus for coagulation factor XIIIA (F13A) is located distal to the HLA region on chromosome 6p in man. *Am J Hum Genet* 1985; **37**: 215-220
16 **Yamanishi K**, Inazawa J, Liew FM, Nonomura K, Ariyama T, Yasuno H, Abe T, Doi H, Hirano J, Fukushima S. Structure of the gene for human transglutaminase 1. *J Biol Chem* 1992; **267**: 17858-17863
17 **Gentile V**, Davies PJ, Baldini A. The human tissue transglutaminase gene maps on chromosome 20q12 by in situ fluorescence hybridization. *Genomics* 1994; **20**: 295-297
18 **Wang M**, Kim IG, Steinert PM, McBride OW. Assignment of the human transglutaminase 2 (TGM2) and transglutaminase 3 (TGM3) genes to chromosome 20q11.2. *Genomics* 1994; **23**: 721-722
19 **Gentile V**, Grant FJ, Porta R, Baldini A. Localization of the human prostate transglutaminase (type IV) gene (TGM4) to chromosome 3p21.33-p22 by fluorescence in situ hybridization. *Genomics* 1995; **27**: 219-220
20 **Grenard P**, Bates MK, Aeschlimann D. Evolution of transglutaminase genes: identification of a transglutaminase gene cluster on human chromosome 15q15. Structure of the gene encoding transglutaminase X and a novel gene family member, transglutaminase Z. *J Biol Chem* 2001; **276**: 33066-33078
21 **Thomázy V**, Fésüs L. Differential expression of tissue transglutaminase in human cells. An immunohistochemical study. *Cell Tissue Res* 1989; **255**: 215-224
22 **Bailey CD**, Johnson GV. Developmental regulation of tissue transglutaminase in the mouse forebrain. *J Neurochem* 2004; **91**: 1369-1379
23 **Kim SY**, Grant P, Lee JH, Pant HC, Steinert PM. Differential expression of multiple transglutaminases in human brain. Increased expression and cross-linking by transglutaminases 1 and 2 in Alzheimer's disease. *J Biol Chem* 1999; **274**: 30715-30721
24 **Citron BA**, SantaCruz KS, Davies PJ, Festoff BW. Intron-exon swapping of transglutaminase mRNA and neuronal Tau aggregation in Alzheimer's disease. *J Biol Chem* 2001; **276**: 3295-3301
25 **De Laurenzi V**, Melino G. Gene disruption of tissue transglutaminase. *Mol Cell Biol* 2001; **21**: 148-155
26 **Lorand L**, Graham RM. Transglutaminases: crosslinking enzymes with pleiotropic functions. *Nat Rev Mol Cell Biol* 2003; **4**: 140-156
27 **Adams RD**, Victor M. Principles of neurology. 5th ed. New York: Mc Graw-Hill, 1993
28 **Selkoe DJ**, Abraham C, Ihara Y. Brain transglutaminase: in vitro crosslinking of human neurofilament proteins into insoluble polymers. *Proc Natl Acad Sci USA* 1982; **79**: 6070-6074
29 **Grierson AJ**, Johnson GV, Miller CC. Three different human tau isoforms and rat neurofilament light, middle and heavy chain proteins are cellular substrates for transglutaminase. *Neurosci Lett* 2001; **298**: 9-12
30 **Singer SM**, Zainelli GM, Norlund MA, Lee JM, Muma NA. Transglutaminase bonds in neurofibrillary tangles and paired helical filament tau early in Alzheimer's disease. *Neurochem Int* 2002; **40**: 17-30
31 **Jeitner TM**, Matson WR, Folk JE, Blass JP, Cooper AJ. Increased levels of gamma-glutamylamines in Huntington disease CSF. *J Neurochem* 2008; **106**: 37-44
32 **Hartley DM**, Zhao C, Speier AC, Woodard GA, Li S, Li Z, Walz T. Transglutaminase induces protofibril-like amyloid beta-protein assemblies that are protease-resistant and inhibit long-term potentiation. *J Biol Chem* 2008; **283**: 16790-16800
33 **Citron BA**, Suo Z, SantaCruz K, Davies PJ, Qin F, Festoff BW. Protein crosslinking, tissue transglutaminase, alternative splicing and neurodegeneration. *Neurochem Int* 2002; **40**: 69-78
34 **Junn E**, Ronchetti RD, Quezado MM, Kim SY, Mouradian MM. 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* 2003; **100**: 2047-2052
35 **Zemaitaitis MO**, Lee JM, Troncoso JC, Muma NA. Transglutaminase-induced cross-linking of tau proteins in progressive supranuclear palsy. *J Neuropathol Exp Neurol* 2000; **59**: 983-989
36 **Zemaitaitis MO**, Kim SY, Halverson RA, Troncoso JC, Lee JM, Muma NA. Transglutaminase activity, protein, and mRNA expression are increased in progressive supranuclear palsy. *J Neuropathol Exp Neurol* 2003; **62**: 173-184
37 **Iuchi S**, Hoffner G, Verbeke P, Djian P, Green H. Oligomeric and polymeric aggregates formed by proteins containing expanded polyglutamine. *Proc Natl Acad Sci USA* 2003; **100**: 2409-2414
38 **Gentile V**, Sepe C, Calvani M, Melone MA, Cotrufo R, Cooper AJ, Blass JP, Peluso G. 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* 1998; **352**: 314-321
39 **Kahlem P**, Green H, Djian P. Transglutaminase action imitates Huntington's disease: selective polymerization of Huntingtin containing expanded polyglutamine. *Mol Cell* 1998; **1**: 595-601
40 **Karpuj MV**, Garren H, Slunt H, Price DL, Gusella J, Becher MW, Steinman L. Transglutaminase aggregates huntingtin into nonamyloidogenic polymers, and its enzymatic activity increases in Huntington's disease brain nuclei. *Proc Natl Acad Sci USA* 1999; **96**: 7388-7393
41 **Segers-Nolten IM**, Wilhelmus MM, Veldhuis G, van Rooijen BD, Drukarch B, Subramaniam V. Tissue transglutaminase modulates alpha-synuclein oligomerization. *Protein Sci* 2008; **17**: 1395-1402
42 **Lai TS**, Tucker T, Burke JR, Strittmatter WJ, Greenberg CS. Effect of tissue transglutaminase on the solubility of proteins containing expanded polyglutamine repeats. *J Neurochem* 2004; **88**: 1253-1260
43 **Konno T**, Morii T, Shimizu H, Oiki S, Ikura K. Paradoxical inhibition of protein aggregation and precipitation by transglutaminase-catalyzed intermolecular cross-linking. *J Biol Chem* 2005; **280**: 17520-17525
44 A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group. *Cell* 1993; **72**: 971-983
45 **Banfi S**, Chung MY, Kwiatkowski TJ Jr, Ranum LP, McCall AE, Chinault AC, Orr HT, Zoghbi HY. Mapping and cloning of the critical region for the spinocerebellar ataxia type 1 gene (SCA1) in a yeast artificial chromosome contig spanning 1.2 Mb. *Genomics* 1993; **18**: 627-635
46 **Sanpei K**, Takano H, Igarashi S, Sato T, Oyake M, Sasaki H, Wakisaka A, Tashiro K, Ishida Y, Ikeuchi T, Koide R, Saito M, Sato A, Tanaka T, Hanyu S, Takiyama Y, Nishizawa M, Shimizu N, Nomura Y, Segawa M, Iwabuchi K, Eguchi I, Tanaka H, Takahashi H, Tsuji S. Identification of the spinocerebellar ataxia type 2 gene using a direct identification of repeat expansion and cloning technique, DIRECT. *Nat Genet* 1996; **14**: 277-284
47 **Pujana MA**, Volpini V, Estivill X. Large CAG/CTG repeat templates produced by PCR, usefulness for the DIRECT method of cloning genes with CAG/CTG repeat expansions. *Nucleic Acids Res* 1998; **26**: 1352-1353
48 **Fletcher CF**, Lutz CM, O'Sullivan TN, Shaughnessy JD Jr, Hawkes R, Frankel WN, Copeland NG, Jenkins NA. Absence epilepsy in tottering mutant mice is associated with calcium channel defects. *Cell* 1996; **87**: 607-617

49 **Vincent JB**, Neves-Pereira ML, Paterson AD, Yamamoto E, Parikh SV, Macciardi F, Gurling HM, Potkin SG, Pato CN, Macedo A, Kovacs M, Davies M, Lieberman JA, Meltzer HY, Petronis A, Kennedy JL. An unstable trinucleotide-repeat region on chromosome 13 implicated in spinocerebellar ataxia: a common expansion locus. *Am J Hum Genet* 2000; **66**: 819-829

50 **Holmes SE**, O'Hearn E, Margolis RL. Why is SCA12 different from other SCAs? *Cytogenet Genome Res* 2003; **100**: 189-197

51 **Imbert G**, Trottier Y, Beckmann J, Mandel JL. The gene for the TATA binding protein (TBP) that contains a highly polymorphic protein coding CAG repeat maps to 6q27. *Genomics* 1994; **21**: 667-668

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

53 **Onodera O**, Oyake M, Takano H, Ikeuchi T, Igarashi S, Tsuji S. Molecular cloning of a full-length cDNA for dentatorubral-pallidoluysian atrophy and regional expressions of the expanded alleles in the CNS. *Am J Hum Genet* 1995; 57: 1050-1060

54 **Cooper AJ**, Sheu KF, Burke JR, Strittmatter WJ, Gentile V, Peluso G, Blass JP. Pathogenesis of inclusion bodies in (CAG)n/Qn-expansion diseases with special reference to the role of tissue transglutaminase and to selective vulnerability. *J Neurochem* 1999; **72**: 889-899

55 **Hadjivassiliou M**, Mäki M, Sanders DS, Williamson CA, Grünewald RA, Woodroofe NM, Korponay-Szabó IR. Autoantibody targeting of brain and intestinal transglutaminase in gluten ataxia. *Neurology* 2006; **66**: 373-377

56 **Boscolo S**, Lorenzon A, Sblattero D, Florian F, Stebel M, Marzari R, Not T, Aeschlimann D, Ventura A, Hadjivassiliou M, Tongiorgi E. Anti transglutaminase antibodies cause ataxia in mice. *PLoS One* 2010; **5**: e9698

57 **Stamnaes J**, Dorum S, Fleckenstein B, Aeschlimann D, Sollid LM. Gluten T cell epitope targeting by TG3 and TG6; implications for dermatitis herpetiformis and gluten ataxia. *Amino Acids* 2010; Epub ahead of print

58 **Griffith OW**, Larsson A, Meister A. Inhibition of gamma-glutamylcysteine synthetase by cystamine: an approach to a therapy of 5-oxoprolinuria (pyroglutamic aciduria). *Biochem Biophys Res Commun* 1977; **79**: 919-925

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. Suppression of aggregate formation and apoptosis by transglutaminase inhibitors in cells expressing truncated DRPLA protein with an expanded polyglutamine stretch. *Nat Genet* 1998; **18**: 111-117

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

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

62 **Lesort M**, Lee M, Tucholski J, Johnson GV. Cystamine inhibits caspase activity. Implications for the treatment of polyglutamine disorders. *J Biol Chem* 2003; **278**: 3825-3830

63 **Hadjivassiliou M**, Aeschlimann P, Strigun A, Sanders DS, Woodroofe N, Aeschlimann D. Autoantibodies in gluten ataxia recognize a novel neuronal transglutaminase. *Ann Neurol* 2008; **64**: 332-343

64 **Krasnikov BF**, Kim SY, McConoughey SJ, Ryu H, Xu H, Stavrovskaya I, Iismaa SE, Mearns BM, Ratan RR, Blass JP, Gibson GE, Cooper AJ. Transglutaminase activity is present in highly purified nonsynaptosomal mouse brain and liver mitochondria. *Biochemistry* 2005; **44**: 7830-7843

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

S- Editor Cheng JX **L- Editor** Lutze M **E- Editor** Zheng XM