Amino Acids (2012) 42:975–986
DOI 10.1007/s00726-011-1011-2

MINIREVIEW ARTICLE

Evidences for a role of protein cross-links in transglutaminase-related disease

Claudio Tabolacci · Alessandro Lentini · Bruno Provenzano · Simone Beninati

Received: 16 March 2011 / Accepted: 24 May 2011 / Published online: 29 July 2011

Abstract Transglutaminases (TGs) are a large family of related and ubiquitous enzymes that catalyze the cross-linking of a glutaminyl residue of a protein/peptide substrate to a lysyl residue of a protein/peptide co-substrate. Considerable and intense progress has been made in the understanding of the chemistry, molecular biology and cell biology of TGs. The knowledge that very different physiological and pathological processes are dependent on the presence of adequate levels of these cross-linking enzymes and on the amount of both free and protein-conjugated polyamines by TG, has generated an incredible amount of original research and review articles. It is clear that TG-mediated reactions are essential for some biological processes, such as blood coagulation, skin barrier formation and extracellular matrix assembly, but may also be involved in pathogenetic mechanisms responsible for several human diseases, such as cancer, AIDS, neurodegenerative disorders, celiac disease, and eye lens opacification. We present here a comprehensive review of recent insights into the pathophysiology of TGs related to their protein cross-linking activity.

Keywords Transglutaminases · Polyamines · Protein cross-link · Post-translational modifications

Abbreviations

TG	Transglutaminase
PUT	Putrescine
SPD	Spermidine
SPM	Spermine
ECM	Extracellular matrix
AD	Alzheimer disease
HD	Huntington disease
PD	Parkinson disease
CSF	Cerebrospinal fluid
HLA	Human leukocyte antigen
CD	Celiac disease
HIV-1 PR	HIV-1 aspartyl protease

C. Tabolacci · A. Lentini · B. Provenzano · S. Beninati (✉)
Department of Biology, University of Rome "Tor Vergata", Via della Ricerca Scientifica, 00133 Rome, Italy
e-mail: beninati@bio.uniroma2.it

Introduction

Transglutaminases (TGs; EC 2.3.2.13) catalyze a calcium-dependent acyl transfer reaction between the γ-carboxamide group of a peptide-bound glutamine residue and the ε-amino group of a peptide-bound lysine, leading to the formation of a ε-(γ-glutamyl)lysine cross-link. Besides, these enzymes can incorporate several low molecular weight amines into proteins in the form of amides of the γ-carboxyl group of a peptide-bound glutamic acid (Folk 1980; Greenberg et al. 1991). TG-catalyzed reactions are extremely specific for a particular glutamine residue in native protein substrates. Because of this extreme specificity, it has been suggested that TG-catalyzed post-translational modification may be physiologically important (Davies et al. 1988). Among the naturally occurring di- and polyamines, putrescine (PUT), spermidine (SPD), and spermine (SPM) are excellent substrates of TG in vitro (Folk et al. 1980; Beninati et al. 1988). The incorporation of these amines into proteins can occur through one or both of their primary amino groups. The result of these reactions is the formation of either *mono*(γ-glutamyl)- or *bis*(γ-glutamyl)-PUT, -SPD or -SPM. Because the K_m of polyamines in the TG reaction in vitro is in the range of concentrations observed in vivo (Williams-

Ashman and Canellakis 1979), it has been suggested that "polyamination" reactions are physiologically relevant. In particular, it has been speculated that polyamine binding to a specific glutamine residue may naturally occur in vivo to modify the structural and/or catalytic properties of a protein (Beninati and Mukherjee 1992; Cordella-Miele et al. 1993; Lentini et al. 2009). Changes in intracellular polyamine levels have been associated with many biological processes, such as cell proliferation and differentiation, embryonic development and neoplastic growth (Heby 1981; Erwin et al. 1984; Fesus and Piacentini 2002). It has been reported that several animal tissues, including rat liver, testis and kidney, human skin and cultured mammalian epidermis cells, contain measurable amounts of (γ-glutamyl)polyamines (Beninati et al. 1984; Beninati and Folk 1988). The levels of these polyamine–protein conjugates, in cells and tissues are correlated with the intracellular TG activity (Davies et al. 1988). Typically, the presence of TG-catalyzed ε(γ-glutamyl)lysine and (γ-glutamyl)polyamine derivatives has been observed in mammalian epidermis. In fact, during terminal differentiation, epidermal cells acquire a deposit of protein on the intracellular surface of the plasma membrane, termed "cornified envelope" (Fig. 1). This cross-linked structure is the most insoluble component of the epidermis, due to disulfide as well as ε(γ-glutamyl)lysine isodipeptide and (γ-glutamyl)polyamine bonds (Martinet et al. 1990). Several proteins, including involucrin, keratolinin and loricrin, are thought to be component of the epidermal envelope, but so far, only loricrin has been shown to be cross-linked to this structure by ε(γ-glutamyl)lysine isodipeptide bonds (Yaffe et al. 1993; Zettergren et al. 1984).

The transglutaminase family

Based on their distinct catalytic characteristic and distribution, several forms of TGs have been identified to date and they have been found to exhibit differences in specificity. These differences are expressed in terms of variations in susceptibility of glutamine residues to catalytic modification and appear to be dependent, at least in part, upon amino acid residues surrounding a given glutamine (Folk and Finlayson 1977). In contrast to their limited glutamine substrate specificity, TGs possess an exceptionally wide specificity for amine substrates. Although the catalytic action of the TGs and their limited specificity are known, much remains to be learned concerning tissue specificity, regulation, and structural relationships. Importantly, all members of the TG superfamily possess a catalytic triad of cysteine 277 (C277), histidine 335 (H335) and aspartate 358 (D358), which is requisite for transamidating

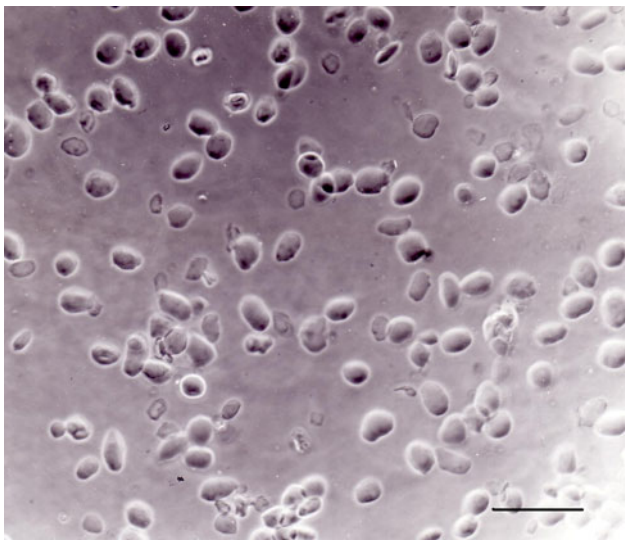

Fig. 1 Cornified envelopes from differentiated mouse epidermal cells. Epidermal cells were prepared from BALB/c mice as described (Piacentini et al. 1988) and cultured under high Ca^{2+} condition (1 mM). *Bar* 100 μm

activity (Liu et al. 2002). TGs are widely distributed in various organs, tissues, and body fluids. They are distinguishable from each other to a large extent by their physical properties and distribution in the body.

Factor XIII is one of the best characterized TG, and its physiological role is well established. It is a plasma protein that circulates in blood as a tetramer of a_2b_2 and consists of two catalytic *a* subunits and two non catalytic *b* subunit (Chung et al. 1974). The *b* subunit is thought to stabilize the *a* subunit. Factor XIII also exists as a dimer of only *a* subunits in platelets, placenta, uterus, prostate, macrophages, and other tissues and cells. To catalyze the covalent cross-linking of blood clots and a number of proteins in plasma, it needs to be activated by thrombin and Ca^{2+} to Factor XIIIa. These include the dimerization of the α chains of two different fibrin molecules followed by the polymerization of the α chains of fibrin (Pisano et al. 1972). These reactions are critical to the blood coagulation cascade and results in the formation of a tough insoluble fibrin clot. A second important reaction catalyzed by Factor XIIIa is the cross-linking of α_2-plasmin inhibitor to the α chains of fibrin (Sakata and Aoki 1980), which plays a significant role in the regulation of fibrinolysis. A third reaction catalyzed by Factor XIIIa is the cross-linking of fibronectin to the α chains of fibrin and to collagen, a reaction closely associated with wound healing (Mosher et al. 1979). Accordingly, a deficiency of Factor XIII can result in a lifelong bleeding tendency, defective wound healing and habitual abortion (Duckert 1972).

A keratinocyte type 1 called TG-K or TG-B (TG-1) activity was identified in cultured epidermal keratinocytes,

rat chondrosarcoma and in epithelial tissues (Kim et al. 1991; Hitomi 2005). TG-1 is synthesized as an 817-residue polypeptide, resulting in a molecular size of approximately 106 kDa. In differentiating keratinocytes, TG-1 shows low specific activity as the zymogen form. During terminal differentiation, TG-1 is proteolyzed into a processed form of 10, 33, and 67 kDa complex that are held together with noncovalent binding. The resulting 10/33/67 kDa complex shows a drastic enhancement of specific activity and is responsible for most of TG-1 activity (Kim et al. 1995). The protease(s) required for activating the zymogen of TG 1 in vivo remains unknown. TG-1 is a key enzyme in the cross-link processes of elafin, filaggrin, loricrin and small proline-rich proteins for the formation and maintenance of cell envelopes (Steinert and Marekov 1995).

Epidermal TG-3 initially designated as an epidermal-type enzyme called TG-E, is responsible for the formation of the epidermis (Kim et al. 1990). It is expressed in the upper epidermal layers and is localized in the cytoplasm (Hitomi et al. 2003). In the current model of TG function, during keratinocyte differentiation, TG-1 and TG-3 are believed to act cooperatively in the cross-linking of proteins, including involucrin, loricrin and small proline-rich proteins. Such concerted reactions result in the formation of the cornified envelope (Candi et al. 2005), a specialized component consisting of covalent crosslinks of proteins beneath the plasma membrane of terminally differentiated keratinocytes. Furthermore, TG-3 in hair follicles is involved in crosslinking structural proteins, such as trichohyalin and keratin intermediate to harden the inner root sheath. In this case, TG-1 cooperates with TGase 3 through a cross-linking reaction to produce stable hair fibers. During differentiation in these processes, a zymogen form of TG-3 (77 kDa) is activated by limited proteolysis with proteases. Under denaturing condition, the enzyme dissociates into two fragments with molecular weights of 50 and 27 kDa, probably linked together by a non-covalent linkage (Kim et al. 1990). Although several studies have focused on the localization, structural analysis and activation mechanism of TG-3 zymogen, not much information is available about the substrate specificity and physiological function of the active form (Ahvazi et al. 2004).

The prostatic secretory type 4 (TG-P) is essential for fertility in rodents. In the prostate, the expression of TG-P is restricted to luminal epithelial cells of the gland. The expression of the TG-P protein could occasionally be observed in high-grade prostatic intraepithelial neoplasia, but was either at a lower level in prostate cancer compared with normal tissues. The expression pattern observed for TG-P in the prostate has not been found thus far for any other prostate-specific marker (Dubbink et al. 1999).

In contrast to the other members of this protein family, tissue TG type 2 (tTG or TG-C) is a multifunctional enzyme apparently involved in very disparate biological processes (Fesus and Piacentini 2002) and its activities are differentially regulated depending on its subcellular localization and may exert differential effects on cell survival. In addition to catalyzing the Ca^{2+}-dependent protein cross-linking reactions, tTG can catalyze Ca^{2+}-independent hydrolysis of GTP and ATP (Lorand and Graham 2003), protein disulfide isomerase reactions (Hasegawa et al. 2003) and serine/threonine kinase activity (Mishra and Murphy 2004). Studies report that tTG is a GTP-binding protein and shows GTPase activity. The cross-linking activity of tTG can be inhibited by GTP. Thus, cellular tTG may function as a multifunctional enzyme, and it would be of interest to determine if tTG has multifunctional activities. Currently, it is well known that tTG is a clear example of product of a single gene involved both in the protection of cellular stress as well as in favoring cell death (Antonyak et al. 2006). The often contradictory cellular function attributed to tTG are apparently confusing, and it rises questions regarding how its transamidation activity might account for such opposing biological outcomes as cell survival and cell death. Several groups have reported the identification of a novel TG RNA transcript whose expression can be induced in cells by cytokines and is detected in the brain of Alzheimer's patients (Monsonego et al. 1997; Citron et al. 2002). Two isoforms of tTG mRNA and protein have been characterized (Fraij et al. 1992; Citron et al. 2002). tTG precursor mRNA is alternatively spliced to generate short-form (tTG-S) mRNA, in addition to a full-length protein (tTG-L). At the protein level, whereas the N-terminal 538 amino acids are shared, tTG-S contain 10 unique amino acids, and tTG-L contains 149 distinctive amino acids at the C-terminus. Structurally, both tTG-L and tTG-S contain the transamidase active site catalytic triad (Cys-277, His-355 and Asp-358); however, compared with tTG-L protein, tTG-S protein lacks the GTP-binding Arg-580 residue. As we know, tTG is a multifunctional enzyme with both GTP binding and Ca^{2+}-activated transamidase activities. GTP binding inhibits transamidation and oppositely transamidation primed by Ca^{2+} represses GTP binding (Begg et al. 2006). By virtue of its reciprocal Ca^{2+}-dependent cross-linking activity, or GTP-dependent signal transducing activity, tTG exhibits multifunctionality at the molecular level, dependent on whether transamidation or GTP binding is switched on, in a mutually exclusive way. The apparent contradictory results published about the role of tTG in neoplastic growth and metastasis (Beninati et al. 1993; Fok et al. 2006; Chhabra et al. 2009; Tabolacci et al. 2010) can be explained considering the two isoforms of tTG in cancer cells. The presence of high levels of Ca^{2+} induces the transamidating

activity of tTG-S isoform, resulting in cell differentiation and death (Beninati et al. 1993). In contrast, low levels of Ca^{2+} and high concentration of GTP induces the function of tTG-L, leading to cell survival, increased invasion and metastatic spread (Chhabra et al. 2009). Although it is predominantly a cytosolic protein, tTG also can be secreted outside the cell and can translocate to the nucleus with the help of importin-α3 protein (Peng et al. 1999), and can be expressed on the cell membrane in association with *β*-members of the integrin family of proteins. Therefore, cell surface tTG promotes adhesion, spreading of cells and enhances focal adhesions (Akimov et al. 2000). We may presume that integrins, in association with tTG-L (the "*glue*") can exert a remarkable positive influence on the migration and proliferation potential of cancer cells, affecting invasion and metastasis. In contrast, the increase in the intracellular levels of Ca^{2+} induces activation of endogenous tTG-S (the "*cross-linking*") with a decisive antiproliferative response, leading to cell differentiation and apoptosis. These findings suggest that activation of endogenous tTG-S may be a useful approach for inducing antineoplastic activity by cell differentiation and apoptosis. This peculiar cross-linking activity can subserve disparate biological phenomena, depending on the location of the target proteins. Depending on cell type and apoptotic stimuli, it can exert a protective role, as well as a facilitory role, on apoptosis (Fesus and Szondy 2005). Furthermore, a growing number of publications show that increased autophagy is often associated with apoptosis induction (Fimia and Piacentini 2010). Deregulation of autophagy has been proposed to be involved in several tTG-associated pathologies (Facchiano et al. 2006). Intracellular activation of tTG can give rise to cross-linked protein envelopes in apoptotic cells, whereas extracellular activation contributes to stabilization of the extracellular matrix (ECM) and promotes cell–substrate interaction (Beninati et al. 1994). Although tTG synthesis and activation is normally part of a protective cellular response contributing to tissue homeostasis, the enzyme has also been implicated in a number of pathological conditions, including fibrosis, atherosclerosis, celiac disease, neurodegenerative diseases and cancer growth and metastasis. Three physiological roles are unequivocally established for TGs: blood coagulation, assembly of a "cornified envelope" in epidermal keratinocytes, apoptotic body formation and origin of the post-ejaculatory vaginal plug by prostate transglutaminase in rodents. Several evidences have been found on additional physiological or pathological functions that transglutaminase may affect. These include irreversible membrane stiffening of erythrocytes, opacification of eye lens, receptor-mediated endocytosis, regulation of cell growth and differentiation, tumor metastasis, programmed cell death and celiac disease.

Involvement of transglutaminase in pathology

Cancer

A field of active research on the role of tTG in human pathology is about neoplastic diseases. It has been observed that cancer cells exhibit a lower tTG transamidating activity, than their normal counterparts (Beninati 1995). It is also noteworthy that induction of tTG activity by powerful natural agents, such as retinoids and methylxanthines leads to an effective switch to cell differentiation and apoptotic death. The observation that retinoid analogs can be even more active in inducing tTG activity and apoptosis in cell lines stimulated further researches (Lentini et al. 2009). The decline of tTG transamidating activity in tumors is potentially a bad prognostic biomarker and is possibly related to tumor metastatic potential, dictating the ability of the cells to cross basal membranes and to invade the bloodstream. Given the proposed functions of tTG, a reduced enzyme expression (Fok et al. 2006) and transamidating activity (Lentini et al. 2009) in tumors would indeed lead to reduced cell adhesion, increased migration and a less stable ECM, thus facilitating the first step of the invasive process by cancer cells. Multiple studies have shown that tTG protein is upregulated in various cancerous tissues (Mehta et al. 2004; Satpathy et al. 2007). In contrast, there are previous reports suggesting that the expression of tTG is downregulated in certain types of cancer (Birckbichler et al. 2000; Jones et al. 2006). Particular attention has been given to the role of the post-translational modification of protein with polyamines in metastasis formation. Evidences for the formation and intracellular localization of *γ*-glutamyl-polyamine derivatives in two murine melanoma cell lines with different metastatic potential have been provided (Beninati et al. 1993). Although the results demonstrated the presence of protein-bound polyamines in these cancer cells, pronounced differences were observed in the two cell lines investigated. Whereas few polyamine conjugates were found in highly metastatic B16-F10 cells, many of those were identified in the lowly metastatic counterparts, B16-$F10^{Lr6}$. The finding of N^1,N^8-*bis*(*γ*-glutamyl)SPD in the proteolytic digest from the less metastatic cell line (B16-$F10^{Lr6}$) suggests a role for this cross-link in the modulation of the metastatic potential of melanoma cells (Fig. 2). Commonly, the levels of free polyamines are higher as compared to the normal counterpart. Therefore, the possibility of increasing intracellular TG transamidating activity and consequently the amount of protein–polyamine conjugates has been considered a promising approach for cancer research. The role of the post-translational modification of ECM and basement membrane proteins with polyamines in the metastatic process, catalyzed by an

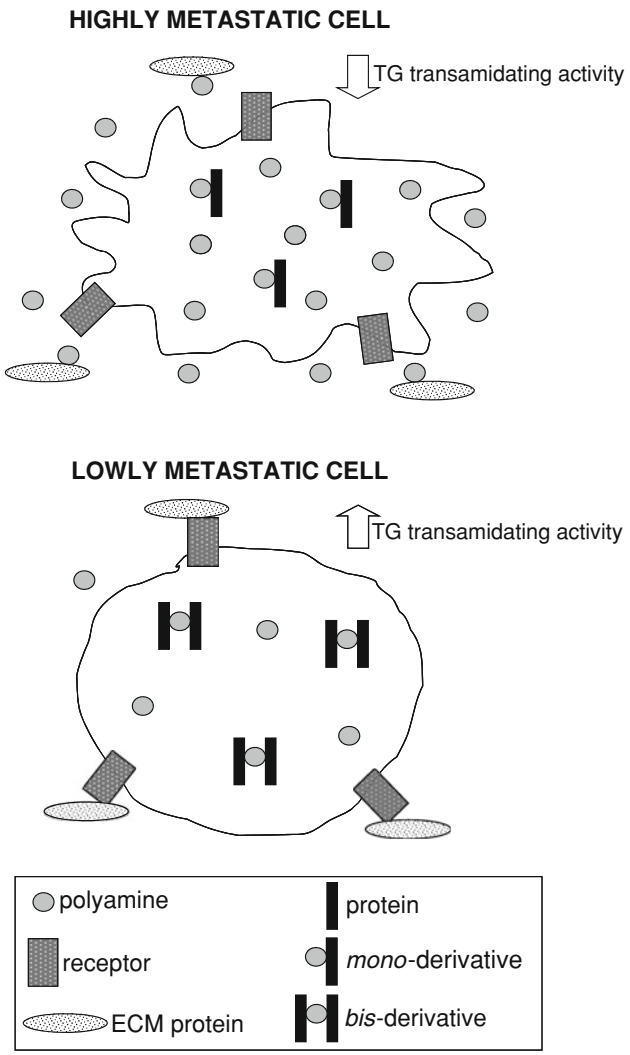

Fig. 2 Involvement of transglutaminase (TG) in the metastatic potential of cancer cells. Highly metastatic cells are characterized by low tTG transamidating activity and high levels of cytoplasmic and extracellular polyamines, which favor the preferential formation of *mono*(γ-glutamyl)derivatives of polyamines. This condition leads to the increase in intracellular plasticity and to an impairment of cell adhesion to the extracellular matrix. In contrast, in lowly metastatic cells, tTG transamidating activity is higher, polyamine content is lowered and protein polymerization increased, through the formation of ε(γ-glutamyl)lysine and *bis*(γ-glutamyl)derivatives of polyamines (Beninati et al. 1993)

activated tTG, has been extensively investigated in B16-F10 murine melanoma cells (Lentini et al. 2008).

In the prostate, the expression of TG-P is restricted to luminal epithelial cells of the gland. The expression of the TG-P protein could occasionally be observed in high-grade prostatic intraepithelial neoplasia, but was either at a lower level in prostate cancer when compared with normal tissues or absence in certain prostate carcinoma cells. The expression pattern observed for TG-P in the prostate has not been found thus far for any other prostate-specific marker (Dubbink et al. 1999). Metastatic prostate tumors also showed loss of expression of TG-P (An et al. 1999). TG-P has a relatively wide profile of expression in human cancer cell lines and is strongly expressed in the low invasive CA-HPV-10 prostate cancer cell line. This enzyme is associated with the invasive potential of prostate cancer cells (Davies et al. 2007). The function of the TG-P is not clear. It has been reported that a 30- and a 100-kDa GTPase are linked to the prostatic secretion of TG-P (Spina et al. 1999). Rat prostate TG (dorsal prostate TG or dorsal protein 1) has been suggested to be responsible for the protein cross-linking during the copulatory plug formation and may be involved to some degree in sperm cell motility and immunogenicity and immunoregulation (Ablin and Whyard 1991). These data, together with the report that TG-P can be up-regulated by androgen in PC-346C, but not in LNCaP cells (despite that both are androgen responsive cell lines) (Dubbink et al. 1996) suggest that the enzyme may also play a role in the control of invasiveness of prostate cancer cells. In conclusion, the relevance of TGs to cancer biology may depend on the type, location, and possibly the stage of the cancer. Therefore, precise understanding of TG functions in context to cancer stage and type is important to implement TG-based therapies.

Neurodegenerative disease

The activity, expression and amounts of individual TG enzymes are increased in a variety of neurodegenerative diseases (Jeitner et al. 2009). TG activity is significantly elevated in the affected cerebral regions in Alzheimer disease (AD) (Kim et al. 1999), Huntington disease (HD) (Karpuj et al. 1999), and supranuclear palsy (Zemaitaitis et al. 2000). These increases in activity are often accompanied by gains in the amount of TG-K and tTG proteins in AD brain (Bonelli et al. 2002), and also of tTG protein in the brains of HD and Parkinson disease (PD) patients (Lesort et al. 1999; Vermes et al. 2004). In addition, the conditions favoring the activation of these enzymes are enhanced in these diseases, such as increase in intracellular Ca^{2+}, due to glutamate-mediated excitotoxicity (Caccamo et al. 2004), or decrease in GTP level (Lin and Beal 2006). Increased TG transamidating activity in neurodegenerative disorders is accompanied by an increase in TG-catalyzed products. Selkoe et al. (1982) demonstrated that cerebral TGs catalyze the in vitro polymerization of cytoskeletal elements, and hypothesized that TGs might facilitate paired helical formation in AD tangles. tTG was also shown to co-localize with plaques in AD brain (Zhang et al. 1998). Components of the plaques, including β-amyloid (Ikura et al. 1993; Ho et al. 1994; Rasmussen et al. 1994), are TG substrates. The in vitro products of the reaction of these substrates with TG bear a striking resemblance to the

insoluble polymers found in AD brain (Jensen et al. 1995; Hartley et al. 2008). HD is caused by a CAG expansion in the huntingtin gene that encodes a length of contiguous Q-residues (polyglutamine) in the N-terminus of the expressed protein. It has been hypothesized that the expanded polyglutamine region would favor the formation of TG-catalyzed huntingtin-containing aggregates. In support of this hypothesis, expanded polyglutamine domains are excellent TG substrates (Gentile et al. 1998; Zainelli et al. 2005). Cellular stresses can trigger the release of huntingtin from the endoplasmic reticulum, allowing huntingtin nuclear entry. Endogenous, full-length huntingtin localizes to nuclear cofilin–actin rods during stress and is required for the proper stress response involving actin remodeling. Mutant huntingtin induces a dominant, persistent nuclear rod phenotype similar to that described in AD for cytoplasmic cofilin–actin rods. The stress response is similarly impaired when mutant huntingtin is present, or when normal huntingtin levels are reduced. Cross-linked complexes of actin and cofilin were found in lymphocyte samples from HD patients, varying in correlation with disease progression. The stress-activated tTG is responsible for the actin–cofilin covalent cross-linking observed in HD (Munsie et al. 2011). These data support a direct role for huntingtin in nuclear actin re-organization, and describe a new pathogenic mechanism for aberrant TG2 enzymatic hyperactivity in neurodegenerative diseases.

As noted earlier, the isopeptide bonds in (γ-glutamyl) amine linkages are resistant to proteolysis (Fink and Folk 1981). Moreover, the ability to metabolize free (γ-glutamyl)amines in brain is limited. Consequently, (γ-glutamyl)SPD, *mono*(γ-glutamyl)PUT and *bis*(γ-glutamyl)PUT are excised intact during proteolysis and are present in brain and cerebrospinal fluid (CSF) (Jeitner et al. 2008). Several authors have raised the possibility that TG inhibitors may be of therapeutic benefit in neurodegenerative diseases (Gentile and Cooper 2004), and one such in vitro inhibitor, cystamine, is beneficial in murine models of PD (Stack et al. 2008). The beneficial effect of tTG inhibition is a compelling argument for the involvement of TGs in neurodegeneration (Mastroberardino et al. 2002). Huntington's disease leads to striatal degeneration via the transcriptional dysregulation of a number of genes. tTG, which is upregulated in HD, exacerbates transcriptional dysregulation by acting as a selective corepressor of nuclear genes (Ballestar et al. 1996). tTG inhibition normalized expression of 40% of genes that are dysregulated in HD striatal neurons, including chaperone and histone genes (McConoughey et al. 2010). In this regard, several groups are actively synthesizing more selective TG inhibitors than cystamine as possible therapeutic agents. These inhibitors include dihydroisoxazole derivatives, peptide-bound 1,2,4-thiadiazoles, peptides containing diazo-5-oxo-l-norleucine in place of glutamine, α, β-unsaturated amides and epoxides (Pardin et al. 2008). Although TGs do not cause neurodegenerative diseases directly, the current evidence suggests that this family of enzymes contributes to the neuropathology once the disease process has begun. Previous work showed that TG activity and free ε(γ-glutamyl)lysine levels are elevated in CSF in patient with HD (Zainelli et al. 2003). Although free ε(γ-glutamyl)lysine was used in these studies as an index of in situ TG activity (γ-glutamyl)polyamines may also be diagnostic. Although these studies focused on the analysis of CSF, a new method, recently described, was also able to detect (γ-glutamyl)polyamines in the plasma, semen, and urine. Interestingly, the (γ-glutamyl)amines were differentially distributed in these fluids. ε(γ-glutamyl)lysine, *mono*(γ-glutamyl)SPD, *mono*(γ-glutamyl)PUT and *bis*(γ-glutamyl)PUT were present in CSF, whereas only ε(γ-glutamyl)lysine and *mono*(γ-glutamyl)PUT were detected in semen. Similarly, ε(γ-glutamyl)lysine and *mono*(γ-glutamyl)SPD were identified in plasma, but not *mono*(γ-glutamyl)PUT or *bis*(γ-glutamyl)PUT (Jeitner et al. 2008). It is anticipated that potent TG inhibitors will soon be evaluated for their therapeutic potential in cellular and animal models of HD and other neurodegenerative diseases. Care will be required to ensure that these inhibitors are sufficiently selective so as not to affect crucial TG reactions critical to normal metabolic processes or to inhibit blood clot formation.

Celiac disease

Celiac disease (CD) is a life-long autoimmune condition of the gastrointestinal tract, affecting the small intestine of genetically susceptible individuals (Lerner et al. 1996). The best characterized genetic factors contributing to disease predisposition are the human leukocyte antigen (HLA) molecules DQ2 and DQ8. Approximately, 95% of patients carry the alleles encoding the DQ2- and most of the rest the DQ8-molecule (Jabri and Sollid 2009). Gluten, which is the storage wheat protein and its alcohol soluble gliadins are the offending inducers of the disease, together with structurally related molecules found in barley and rye. tTG is the autoantigen against which the abnormal immune response is directed to (Reif and Lerner 2004) and two main autoantibodies (anti-endomysium and anti-tTG) are the most useful serological markers for the disease screening (Shamir et al. 2002; Lindfors et al. 2010). tTG activity has been accurately investigated in CD research, because its identification as the major autoantigen targeted by the disease (Dieterich et al. 1997). During the pathogenesis of CD, tTG is able to deamidate specific glutamine residues in immunogenetic gliadin peptides, creating epitopes which bind to DQ2 with increased affinity (Molberg et al. 1998). Moreover, the tTG-modified gliadin peptides

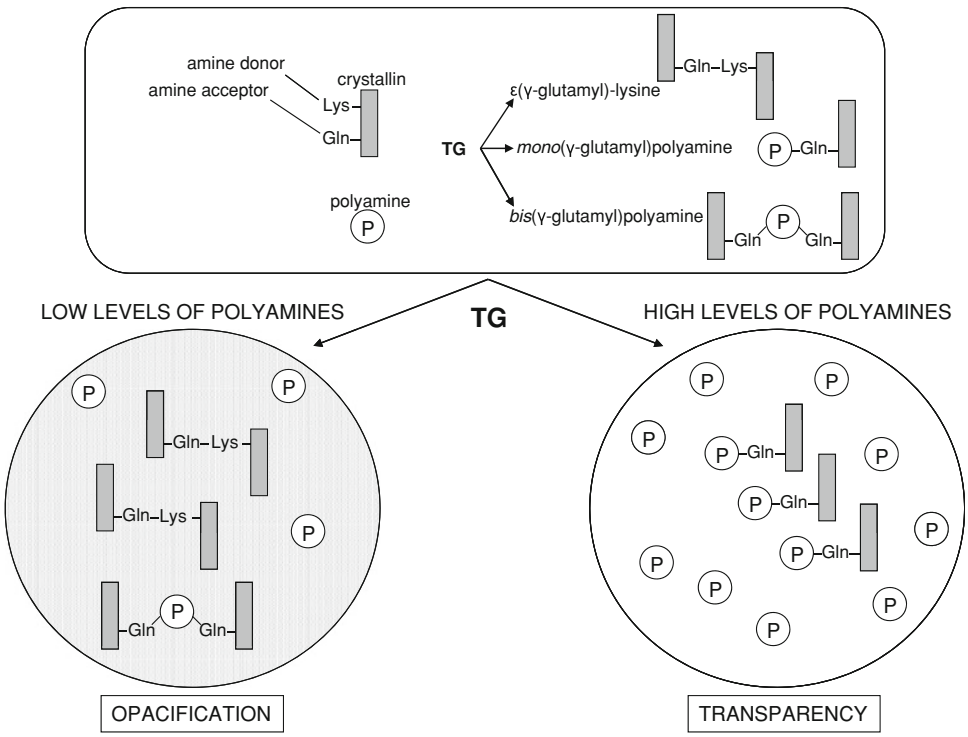

Fig. 3 Possible role of lens polyamines as regulators of endogenous transglutaminase (TG) activity and eye lens opacification. Low levels of endogenous polyamines may create the suitable condition for irreversible polymerization of crystallins, by ε(γ-glutamyl)lysine and/or *bis*(γ-glutamyl)derivatives of polyamines, inducing lens opacification. Conversely, crystallin polymerization may be prevented in the presence of high concentration of polyamines. In this condition, all the available reactive glutaminyl residues are sequestered by polyamines as *mono*(γ-glutamyl)derivatives, maintaining lens transparency (Lentini et al. 2011)

are efficiently recognized by activated T cells derived from CD patients (Shan et al. 2002). Furthermore, tTG is able to cross-link gliadin peptides to several ECM proteins, as well as itself leading to the accumulation of gliadin peptides in the lamina propria favoring the progression of CD (Skovbjerg et al. 2004). Cross-linking also occurs outside the active site of tTG and results in permanently and covalently linked deamidated gliadin peptide/tTG complexes (Fleckenstein et al. 2004). It remains poorly understood how the autoantibodies to tTG develop (Dewar et al. 2004) although it has been postulated that the formation of the complex of gliadin peptides and tTG is a hint supporting epitope spreading from gliadin to tTG.

Eye lens opacification

Cataract results from the deposition of aggregated proteins in the eye which causes clouding of the lens, light scattering, and obstruction of vision. Eye lens opacification results from the aberrant cross-linking of predominantly β-crystallins (Sharma and Santhoshkumar 2009) as well as their proteolysis catalyzed by calpains (Biswas et al. 2004). There are a number of compelling observations indicating a pivotal role for lens tTG in cataract development (Lorand et al. 1981; Kremzner et al. 1983), evidences associated with a marked elevation of intracellular Ca^{2+} ions during cataract formation, which activate both tTG and calpains. Other ionic disturbances also occur, such as increases/decreases in $Na^{+,}$ K^{+} and Ca^{2+}, but Ca^{2+} is considered to be the major contributor to cataractogenesis (Fein et al. 1979). The involvement of tTG-catalyzed crosslinking of crystallins in the loss of transparency of cataractous lens has been established (Boros et al. 2008; Lentini et al. 2011). In lens, β-crystallins are the primary targets for tTG-catalyzed cross-linking. Among these, βB2-, βB3- and βA3-crystallins have been identified as potent glutamine substrates for tTG (Lorand et al. 1985), but βA3-, together with βB1- and α-crystallin also expose lysine-donor sites for TG (Groenen et al. 1994). It has also been observed that the rate of crystallins cross-linking operated by tTG may be affected by polyamine levels, modulating the ratio between *mono*- and *bis*(γ-glutamyl)derivatives content (Fig. 3). In fact, high levels of polyamines favor the formation of *mono*-derivatives, due to the rapid saturation of the reactive glutaminyl residues available, which cannot be involved in the formation of protein cross-links [ε(γ-glutamyl)lysine or *bis*(γ-glutamyl)derivatives of polyamines]. In contrast, low levels of endogenous polyamines allow the preferential formation of *bis*(γ-glutamyl)polyamines and/or ε(γ-glutamyl)lysine, creating the suitable condition for irreversible protein polymerization (Lentini et al. 2011).

AIDS and human immunodeficiency virus type 1

The human immunodeficiency virus type-1 (HIV-1) env glycoproteins are synthesized as a precursor (gp160) that is cleaved to generate the surface (gp120) and transmembrane

Table 1 Levels of *mono*- and *bis*-derivatives of SPD and ε(γ-glutamyl)lysine in HIV-1 PR after TG incubation

	Control	TG	TG + 0.1 mM SPD	TG + 1 mM SPD
ε(γ-glutamyl)lysine	15.51 ± 1.30	85.22 ± 7.78*	44.04 ± 4.05**	23.51 ± 2.29**
N^1(γ-glutamyl)SPD	0.54 ± 0.04	0.45 ± 0.02	9.65 ± 0.86**	15.90 ± 1.55**
N^8(γ-glutamyl)SPD	0.33 ± 0.02	0.47 ± 0.04	26.75 ± 2.30**	51.22 ± 4.80**
N^1,N^8-*bis*(γ-glutamyl)SPD	0.40 ± 0.03	0.32 ± 0.02	1.60 ± 0.22**	0.67 ± 0.01**

Values are expressed as nmol/mg protein. The data represent the mean ± SD of three different determinations

From Lentini et al. (2010)

HIV-1 PR HIV-1 aspartyl protease, *TG* transglutaminase, *SPD* spermidine

* Significantly different from control ($p < 0.001$)

** Significantly different from TG ($p < 0.001$)

(gp41) env proteins, which are non-covalently associated with each other (Montagnier et al. 1985). gp120 contains the CD4-binding domains, while gp41 anchors the gp120–gp41 complex in the viral env or host cell membrane. Not much is known either on the molecular mechanisms about the interaction of gp120 with CD4, or other HIV receptors occurring on CD4− cells, and the molecular mechanisms of gp41 anchorage to the cellular membranes. It has been reported that gp41 is not only able to act as a TG amino acceptor, but also as an amino donor substrate and, thus, it could crosslink to receptor(s) occurring on HIV-target cells and/or gp120 with both glutaminyl and lysyl residues (Mariniello et al. 1993a, b).

The HIV-l aspartyl protease (HIV-1 PR) is required for the processing of the viral polyproteins encoded by the gaq and pal genes into mature virion proteins. This processing involves cleavage of the qaq precursor (∼55) to form the four structural proteins of the virion core. Furthermore, processing of the ∼160 gag-pol precursor yields these structural proteins as well as HIV-1 PR, reverse transcriptase and integration protein, essential for HIV replication. The expression of HIV-1 PR in *Escherichia coli* and its subsequent characterization showed that it belongs to the family of aspartyl proteases and that in its active form the enzyme is a homodimer (Wlodawer et al. 1989).

HIV-1 PR was shown to act in vitro as acyl-donor and acyl-acceptor for both guinea pig liver transglutaminase and human Factor XIIIa (Beninati and Mukherjee 1992). These preliminary evidences suggested that the HIV-1 PR contains at least three tTG-reactive glutaminyl and one lysyl residues. We have recently reported that the incubation of HIV-1 PR with tTG increases its catalytic activity (Lentini et al. 2010). This increase is dependent on the time of incubation, the concentration of TG and the presence of Ca^{2+}. Identification of ε(γ-glutamyl)lysine in the proteolytic digest of the TG-modified HIV-1 PR suggested intramolecular covalent cross-linking of this protease, which may promote a non-covalent dimerization and subsequent activation of this enzyme via a conformational change. This hypothesis is supported by the observation that the TG-catalyzed activation of HIV-1 PR was completely abolished by SPD which acts as a competitive inhibitor of ε(γ-glutamyl)lysine formation. Indeed, in the presence of SPD, the formation of the isopeptide was decreased. The main products of the TG-catalyzed modification of HIV-1 PR in the presence of SPD were both *mono*(γ-glutamyl)SPD analogs. Accordingly, negligible amount of N^1,N^8-*bis*(γ-glutamyl)SPD was found (Table 1).

The finding that HIV-1 PR activity can be increased by tTG is intriguing. Because the HIV-1 PR is enzymatically active upon dimerization (Ishima et al. 2001), the transamidation reaction could likely occur in a way similar to that suggested for porcine pancreatic phospholipase A2 (Cordella-Miele et al. 1990, 1993). These findings reveal the presence of a novel enzymatic mechanism by which the slow auto-activation step may be bypassed, or accelerated, through a tTG-mediated post-translational modification of HIV-1 PR. tTG may convert a slowly auto-activating HIV-1 PR into a pre-activated or rapidly auto-activating form with enhanced enzymatic activity, hypothesis supported by previous published results on the role of tTG in the HIV virion assembly (Bergamini et al. 1994). The modification of HIV-1 PR by tTG may be a novel area of enquire for the development of anti-HIV agents. Several lines of evidence have demonstrated the involvement of tTG in HIV pathogenesis (Amendola et al. 2002; Nardacci et al. 2005); nevertheless, the possible role of this post-translational modification in the progression of HIV-1 infection deserves a thorough investigation.

Conclusive remarks

Transglutaminases have been shown to be involved in cataract development, gluten sensitivity diseases, neurodegeneration, and tissue remodeling associated with cancer and wound repair. The transamidase activity of tTG plays a critical role in the formation of lens protein aggregates. The

extracellular transamidase and deamidase activities of tTG are central to the adaptive immune response that is elicited in gluten sensitivity diseases. Whereas tTG-mediated cross-linking of amyloid-forming proteins may prevent Parkinson's disease progression, tTG may contribute to the pathogenesis of Alzheimer's disease and Huntington's disease.

The role of the short, pro-apoptotic, tTG-S isoform in neurodegenerative disease progression and cancer remains to be investigated. The transamidase activity of tTG contributes to extracellular matrix stabilization of host matrix proteins that may retard tumor progression. Extracellularly, and independently of its cross-linking activity, the isoform tTG-L promotes cell adhesion during tissue remodeling by stabilizing the ECM and by interacting with cell surface adhesion receptors, such as integrins. tTG-L also functions intracellularly to regulate cell spreading and motility independent of its cross-linking activity. However, the specific biochemical mechanisms involved in modulating these responses require further characterization. The diverse intracellular and extracellular roles of tTG and the many proteins with which they interact, indicate an interaction between TGs and matrix, receptor, cytosolic, and nuclear proteins. Future studies delineating signaling pathways are needed to link specific effects with a specific TG biochemical function and localization.

Indeed, a key area of research, about which little is currently known, is how tTG is secreted from the cell to the ECM. Further dissection of the overlapping or opposing roles of tTG would benefit from the use of tissue-specific, temporal-specific, or double-knockout mouse models. Finally, a major challenge will be the development, and temporal analysis in animal models, of specific pharmacological inhibitors of transamidase activity for efficacious treatment of cataracts, gluten sensitivity, neurodegenerative diseases and cancer.

References

Ablin RJ, Whyard TC (1991) Identification and biological relevance of spermatozoal transglutaminase. Experientia 47:277–279

Ahvazi B, Boeshans KM, Rastinejad F (2004) The emerging structural understanding of transglutaminase 3. J Struct Biol 147:200–207

Akimov SS, Krylov D, Fleischman LF, Belkin AM (2000) Tissue transglutaminase is an integrin-binding adhesion coreceptor for fibronectin. J Cell Biol 148:825–838

Amendola A, Fesus L, Piacentini M, Szondy Z (2002) "Tissue" transglutaminase in AIDS. J Immunol Methods 265:145–159

An G, Meka CS, Bright S, Veltri RW (1999) Human prostate-specific transglutaminase gene: promoter cloning, tissue-specific expression, and down-regulation in metastatic prostate cancer. Urology 54:1105–1111

Antonyak MA, Jansen JM, Miller AM, Ly TK, Endo M, Cerione RA (2006) Two isoforms of tissue transglutaminase mediate opposing cellular fates. Proc Natl Acad Sci USA 103:18609–18614

Ballestar E, Abad C, Franco L (1996) Core histones are glutaminyl substrates for tissue transglutaminase. J Biol Chem 271:18817–18824

Begg GE, Carrington L, Stokes PH, Matthews JM, Wouters MA, Husain A, Lorand L, Iismaa SE, Graham RM (2006) Mechanism of allosteric regulation of transglutaminase 2 by GTP. Proc Natl Acad Sci USA 103:19683–19688

Beninati S (1995) Post-translational modification of protein in cancer cells: the transglutaminase-catalyzed reaction (editorial). Cancer J 8:234–236

Beninati S, Folk JE (1988) Covalent polyamine-protein conjugates: analysis and distribution. Adv Exp Med Biol 250:411–422

Beninati S, Mukherjee AB (1992) A novel transglutaminase-catalyzed posttranslational modification of HIV-1 aspartyl protease. Biochem Biophys Res Commun 187:1211–1218

Beninati S, Piacentini M, Argento-Cerù MP, Russo-Caia S, Autuori F (1984) Presence of di- and polyamines covalently bound to protein in rat liver. Biochim Biophys Acta 841:120–126

Beninati S, Martinet N, Folk JE (1988) High-performance liquid chromatographic method for the determination of epsilon-(gamma-glutamyl)lysine and *mono*- and *bis*-gamma-glutamyl derivatives of putrescine and spermidine. J Chromatogr 443: 329–335

Beninati S, Abbruzzese A, Cardinali M (1993) Differences in the post-translational modification of proteins by polyamines between weakly and highly metastatic B16 melanoma cells. Int J Cancer 53:792–797

Beninati S, Senger DR, Cordella-Miele E, Mukherjee AB, Chackal-aparampil I, Shanmugam V, Singh K, Mukherjee BB (1994) Osteopontin: its transglutaminase-catalyzed posttranslational modifications and cross-linking to fibronectin. J Biochem 115:675–682

Bergamini A, Capozzi M, Ghibelli L, Salanitro A, Milanese G, Wagner T, Beninati S, Pesce CD, Amici C, Rocchi G (1994) Cystamine potently suppresses in vitro HIV replication in acutely and chronically infected human cells. J Clin Invest 93:2251–2257

Birckbichler PJ, Bonner RB, Hurst RE, Bane BL, Pitha JV, Hamstreet JP (2000) Loss of tissue transglutaminase as a biomarker for prostate adenocarcinoma. Cancer 89:412–423

Biswas S, Harris F, Dennison S, Singh J, Phoenix DA (2004) Calpains: targets of cataract prevention? Trends Mol Med 10:78–84

Bonelli RM, Aschoff A, Niederwieser G, Heuberger C, Jirikowski G (2002) Cerebrospinal fluid tissue transglutaminase as a biochemical marker for Alzheimer's disease. Neurobiol Dis 11: 106–110

Boros S, Wilmarth PA, Kamps B, de Jong WW, Bloemendal H, Lampi K, Boelens WC (2008) Tissue transglutaminase catalyzes the deamidation of glutamines in lens betaB(2)- and betaB(3)-crystallins. Exp Eye Res 86:383–393

Caccamo D, Campisi A, Curro M, Li Volti G, Vanella A, Ientile R (2004) Excitotoxic and post-ischemic neurodegeneration: involvement of transglutaminases. Amino Acids 27:373–379

Candi E, Schmidt R, Melino G (2005) The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 6:328–340

Chhabra A, Verma A, Mehta K (2009) Tissue transglutaminase promotes or suppresses tumors depending on cell context. Anticancer Res 29:1909–1919

Chung SI, Lewis MS, Folk JE (1974) Relationships of the catalytic properties of human plasma and platelet transglutaminases (activated blood coagulation Factor XIII) to their subunit structures. J Biol Chem 249:940–950

Citron BA, Suo Z, SantaCruz K, Davies PJ, Qin F, Festoff BW (2002) Protein crosslinking, tissue transglutaminase, alternative splicing and neurodegeneration. Neurochem Int 40:69–78

Cordella-Miele E, Miele L, Mukherjee AB (1990) A novel transglutaminase mediated post-translational modification of phospholipase A2 dramatically increases its catalytic activity. J Biol Chem 265:17180–17188

Cordella-Miele E, Miele L, Beninati S, Mukherjee AB (1993) Transglutaminase-catalyzed incorporation of polyamines into phospholipase A2. J Biochem 113:164–173

Davies PJA, Chiocca EA, Basilion JP, Poddar S, Stein JP (1988) Transglutaminases and their regulation: implications for polyamine metabolism. Adv Exp Med Biol 250:391–401

Davies G, Ablin RJ, Mason MD, Jiang WG (2007) Expression of the prostate transglutaminase (TGase-4) in prostate cancer cells and its impact on the invasiveness of prostate cancer. J Exp Ther Oncol 6:257–264

Dewar D, Pereira SP, Ciclitira PJ (2004) The pathogenesis of coeliac disease. Int J Biochem Cell Biol 36:17–24

Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO et al (1997) Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 3:797–801

Dubbink HJ, Verkaik NS, Faber PW, Trapman J, Schroder FH, Romijn JC (1996) Tissue-specific and androgen-regulated expression of human prostate-specific transglutaminase. Biochem J 315:901–908

Dubbink HJ, Hoedemaeker RF, van der Kwast TH, Schroder F, Romijn JC (1999) Human prostate-specific transglutaminase: a new prostatic marker with a unique distribution pattern. Lab Invest 79:141–150

Duckert (1972) Documentation of the plasma Factor XIII deficiency in man. Ann NY Acad Sci 202:190–199

Erwin BG, Bethell DR, Pegg AE (1984) Role of polyamines in differentiation of 3T3–L1 fibroblasts into adipocytes. Am J Physiol 246:C293–C300

Facchiano F, Facchiano A, Facchiano AM (2006) The role of transglutaminase-2 and its substrates in human diseases. Front Biosci 11:1758–1773

Fein T, Pande A, Spector A (1979) Further investigation of the role of calcium in human lens protein aggregation. Invest Ophthalmol Vis Sci 18:761–765

Fesus L, Piacentini M (2002) Transglutaminase 2: an enigmatic enzyme with diverse functions. Trends Biochem Sci 27:534–539

Fesus L, Szondy Z (2005) Transglutaminase 2 in the balance of cell death and survival. FEBS Lett 579:3297–3302

Fimia GM, Piacentini M (2010) Regulation of autophagy in mammals and its interplay with apoptosis. Cell Mol Life Sci 67:1581–1588

Fink ML, Folk JE (1981) γ-Glutamylamine cyclotransferase. An enzyme involved in the catabolism of ε-(γ-glutamyl)lysine and other γ-glutamylamines. Mol Cell Biochem 38:59–67

Fleckenstein B, Qiao SW, Larsen MR, Jung G, Roepstorff P, Sollid LM (2004) Molecular characterization of covalent complexes between tissue transglutaminase and gliadin peptides. J Biol Chem 279:17607–17616

Fok JY, Ekmekcioglu S, Mehta K (2006) Implications of tissue transglutaminase expression in malignant melanoma. Mol Cancer Ther 5(6):1493–1503

Folk JE (1980) Transglutaminases. Ann Rev Biochem 49:517–531

Folk JE, Finlayson JS (1977) The epsilon-(gamma-glutamyl)lysine crosslink and the catalytic role of transglutaminases. Adv Protein Chem 31:1–133

Folk JE, Park MH, Chung SI, Schrode J, Lester EP, Cooper HL (1980) Polyamines as physiological substrates for transglutaminases. J BiolChem 255:3695–3700

Fraij BM, Birckbichler PJ, Patterson MK Jr, Lee KN, Gonzales RA (1992) A retinoic acid-inducible mRNA from human erythroleukemia cells encodes a novel tissue transglutaminase homologue. J Biol Chem 267:22616–22623

Gentile V, Cooper AJL (2004) Transglutaminases—possible drug targets in human diseases. Curr Drug Targets CNS Neurol Disord 3:99–104

Gentile V, Sepe C, Calvani M, Melone MA, Cotrufo R, Cooper AJL, 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

Greenberg CS, Birckbichler PJ, Rice RH (1991) Transglutaminases: multifunctional cross-linking enzymes that stabilize tissues. FASEB J 5:3071–3077

Groenen PJ, Grootjans JJ, Lubsen NH, Bloemendal H, de Jong WW (1994) Lys-17 is the amine-donor substrate site for transglutaminase in beta A3-crystallin. J Biol Chem 269:831–833

Hartley DM, Zhao C, Speier AC, Woodard GA, Li S, Li Z, Walz T (2008) Transglutaminase induces protofibril-like amyloid beta-protein assemblies that are protease-resistant and inhibit long-term potentiation. J Biol Chem 283:16790–16800

Hasegawa G, Suwa M, Ichikawa Y, Ohtsuka T, Kumagai S, Kikuchi M, Sato Y, Saito Y (2003) A novel function of tissue-type transglutaminase: protein disulphide isomerase. Biochem J 373:793–803

Heby O (1981) Role of polyamines in the control of cell proliferation and differentiation. Differentiation 21:1–20

Hitomi K (2005) Transglutaminases in skin epidermis. Eur J Dermatol 15:313–319

Hitomi K, Presland RB, Nakayama T, Fleckman P, Dale BA, Maki M (2003) Analysis of epidermal-type transglutaminase (transglutaminase 3) in human stratified epithelia and cultured keratinocytes using monoclonal antibodies. J Dermatol Sci 32:95–103

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

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

Ishima R, Ghirlando R, Tözsér J, Gronenborn AM, Torchia DA, Louis JM (2001) Folded monomer of HIV-1 protease. J Biol Chem 276:49110–49116

Jabri B, Sollid LM (2009) Tissue-mediated control of immunopathology in coeliac disease. Nat Rev Immunol 9:858–870

Jeitner TM, Matson WR, Folk JE, Blass JP, Cooper AJL (2008) Increased levels of gamma-glutamylamines in Huntington disease. J Neurochem 106:37–44

Jeitner TM, Pinto JT, Krasnikov BF, Horswill M, Cooper AJ (2009) Transglutaminases and neurodegeneration. J Neurochem 109:160–166

Jensen PH, Sorensen ES, Petersen TE, Gliemann J, Rasmussen LK (1995) Residues in the synuclein consensus motif of the alpha-synuclein fragment, NAC, participate in transglutaminase-catalysed cross-linking to Alzheimer-disease amyloid β A4 peptide. Biochem J 310:91–94

Jones RA, Kotsakis P, Johnson TS, Chau DY, Ali S, Melino G, Griffin M (2006) Matrix changes induced by transglutaminase 2 lead to inhibition of angiogenesis and tumor growth. Cell Death Differ 13:1442–1453

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

Kim HC, Lewis MS, Gorman JL, Park SC, Giratd JE, Folk JE, Chung SI (1990) Protransglutaminase E from guinea pig skin. Isolation and partial characterization. J Biol Chem 265:21971–21978

Kim HC, Idler WW, Kim GI, Han HJ, Chung SI, Steinert PM (1991) The complete amino acid sequence of the human transglutaminase K enzyme deduced from the nucleic acid sequences of cDNA clones. J Biol Chem 266:536–539

Kim SY, Chung SI, Steinert PM (1995) Highly active soluble processed forms of the transglutaminase 1 enzyme in epidermal keratinocytes. J Biol Chem 270:18026–18035

Kim SY, Grant P, Lee JH, Pant HC, Steinert PM (1999) 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 274:30715–30721

Kremzner LT, Roy D, Spector A (1983) Polyamines in normal and cataractous human lenses: evidence for post-translational modification. Exp Eye Res 37:649–659

Lentini A, Provenzano B, Caraglia M, Shevchenko A, Abbruzzese A, Beninati S (2008) Impairment of the metastatic activity of melanoma cells by transglutaminase-catalyzed incorporation of polyamines into laminin and Matrigel. Amino Acids 34:251–256

Lentini A, Provenzano B, Tabolacci C, Beninati S (2009) Protein–polyamine conjugates by transglutaminase 2 as potential markers for antineoplastic screening of natural compounds. Amino Acids 36:701–708

Lentini A, Tabolacci C, Melino S, Provenzano B, Beninati S (2010) Post-translational modification of glutamine and lysine residues of HIV-1 aspartyl protease by transglutaminase increases its catalytic activity. Biochem Biphys Res Commun 393:546–550

Lentini A, Tabolacci C, Mattioli P, Provenzano B, Beninati S (2011) Spermidine delays eye lens opacification in vitro by suppressing transglutaminase-catalyzed crystallin crosslinking. Protein J 30:109–114

Lerner A, Blank M, Shoenfeld Y (1996) Celiac disease and autoimmunity. Isr J Med Sci 32:33–36

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

Lin MT, Beal MF (2006) Mitochondrial dysfunction and oxidative stress in neurodegenerative diseases. Nature 443:787–795

Lindfors K, Mäki M, Kaukinen K (2010) Transglutaminase 2-targeted autoantibodies in celiac disease: pathogenetic players in addition to diagnostic tools? Autoimmun Rev 9:744–749

Liu S, Cerione RA, Clardy J (2002) Structural basis for the guanine nucleotide-binding activity of tissue transglutaminase and its regulation of transamidation activity. Proc Natl Acad Sci USA 99:2743–2747

Lorand L, Graham RM (2003) Transglutaminases: crosslinking enzymes with pleiotropic functions. Nat Rev Mol Cell Biol 4:140–156

Lorand L, Hsu LKH, Siefring GE Jr, Rafferty NS (1981) Lens transglutaminase and cataract formation. Proc Natl Acad Sci USA 78:1356–1360

Lorand L, Conrad SM, Velasco PT (1985) Formation of a 55 000-weight cross-linked beta crystallin dimer in the Ca^{2+}-treated lens. A model for cataract. Biochemistry 24:1525–1531

Mariniello L, Esposito C, Gentile V, Porta R (1993a) Transglutaminase covalently incorporates amines into human immunodeficiency virus envelope glycoprotein gp120 in vitro. Int J Pept Protein Res 42:204–206

Mariniello L, Esposito C, Di Pierro P, Cozzolino A, Pucci P, Porta R (1993b) Human-immunodeficiency-virus transmembrane glycoprotein gp41 is an amino acceptor and donor substrate for transglutaminase in vitro. Eur J Biochem 215:99–104

Martinet N, Beninati S, Nigra TP, Folk JE (1990) N1N8-*bis*(gamma-glutamyl)spermidine cross-linking in epidermal-cell envelopes. Comparison of cross-link levels in normal and psoriatic cell envelopes. Biochem J 271:305–308

Mastroberardino PG, Iannicola C, Nardacci R et al (2002) 'Tissue' transglutaminase ablation reduces neuronal death and prolongs survival in a mouse model of Huntington's disease. Cell Death Differ 9:873–880

McConoughey SJ, Basso M, Niatsetskaya ZV, Sleiman SF, Smirnova NA, Langley BC et al (2010) Inhibition of transglutaminase 2 mitigates transcriptional dysregulation in models of Huntington disease. EMBO Mol Med 2:349–370

Mehta K, Fok J, Miller FR, Koul D, Sahin AA (2004) Prognostic significance of tissue transglutaminase in drug resistant and metastatic breast cancer. Clin Cancer Res 10:8068–8076

Mishra S, Murphy LJ (2004) Tissue transglutaminase has intrinsic kinase activity: identification of transglutaminase 2 as an insulin-like growth factor-binding protein-3 kinase. J Biol Chem 279:23863–23868

Molberg O, Mcadam SN, Korner R, Quarsten H, Kristiansen C, Madsen L et al (1998) Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. Nat Med 4:713–717

Monsonego A, Shani Y, Friedmann I, Paas Y, Eizenberg O, Schwartz M (1997) Expression of GTP-dependent and GTP-independent tissue-type transglutaminase in cytokine-treated rat brain astrocytes. J Biol Chem 272:3724–3732

Montagnier L, Clavel F, Krust B, Chamaret S, Rey F, Barre-Sinoussi F, Cherman JC (1985) Identification and antigenicity of the major envelope glycoprotein of lymphadenopathy-associated virus. Virology 144:283–289

Mosher DF, Schad PE, Kleinman HK (1979) Cross-linking of fibronectin to collagen by blood coagulation Factor XIIIa. J Clin Invest 64:781–787

Munsie L, Caron N, Atwal RS, Marsden I, Wild EJ, Bamburg JR et al (2011) Mutant huntingtin causes defective actin remodeling during stress: defining a new role for transglutaminase 2 in neurodegenerative disease. Hum Mol Genet 20:1937–1951

Nardacci R, Antinori A, Larocca LM, Arena V, Amendola A, Perfettini JL, Kroemer G, Piacentini M (2005) Characterization of cell death pathways in human immunodeficiency virus-associated encephalitis. Am J Pathol 167:695–704

Pardin C, Pelletier JN, Lubell WD, Keillor JW (2008) Cinnamoyl inhibitors of tissue transglutaminase. J Org Chem 73:5766–5775

Peng X, Zhang Y, Zhang H, Graner S, Williams JF, Levitt ML, Lokshin A (1999) Interaction of tissue transglutaminase with nuclear transport protein importin-alpha3. FEBS Lett 446:35–39

Piacentini M, Martinet N, Beninati S, Folk JE (1988) Free and protein-conjugated polyamines in mouse epidermal cells. Effect of high calcium and retinoic acid. J Biol Chem 263:3790–3794

Pisano JJ, Bronzert TJ, Peyton MP, Finlayson JS (1972) Epsilon(gamma–glutamyl)lysine cross-links: determination in fibrin from normal and Factor XIII-deficient individuals. Ann N Y Acad Sci 202:98–113

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 α 2M receptor. FEBS Lett 338:161–166

Reif S, Lerner A (2004) Tissue transglutaminase—the key player in celiac disease: a review. Autoimmun Rev 3:40–45

Sakata Y, Aoki NM (1980) Cross-linking of alpha 2-plasmin inhibitor to fibrin-stabilizing factor. J Clin Invest 65:290–297

Satpathy M, Cao L, Pincheira R, Emerson R, Bigsby R, Nakshatri H, Matei D (2007) Enhanced peritoneal ovarian tumor dissemination by tissue transglutaminase. Cancer Res 67:7194–7202

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

Shamir R, Eliakim R, Lahat N, Sobel E, Lerner A (2002) ELISA assay of anti endomysial antibodies in the diagnosis of celiac disease: comparison with immunofluorescence assay of anti endomysial antibodies and tissue transglutaminase antibodies. Isr Med Assoc J 4:594–596

Shan L, Molberg O, Parrot I, Hausch F, Filiz F, Gray GM et al (2002) Structural basis for gluten intolerance in celiac sprue. Science 297:2275–2279

Sharma KK, Santhoshkumar P (2009) Lens aging: effects of crystallins. Biochim Biophys Acta 1790:1095–1108

Skovbjerg H, Koch C, Anthonsen D, Sjöström H (2004) Deamidation and cross-linking of gliadin peptides by transglutaminases and the relation to celiac disease. Biochim Biophys Acta 1690:220–230

Spina AM, Esposito C, Pagano M, Chiosi E, Mariniello L, Cozzolino A, Porta R, Illiano G (1999) GTPase and transglutaminase are associated in the secretion of the rat anterior prostate. Biochem Biophys Res Commun 260:351–356

Stack EC, Ferro JL, Kim J et al (2008) Therapeutic attenuation of mitochondrial dysfunction and oxidative stress in neurotoxin models of Parkinson's disease. Biochim Biophys Acta 1782:151–162

Steinert PM, Marekov LN (1995) The proteins elafin, filaggrin, keratin intermediate filaments, loricrin and small pralinerich proteins 1 and 2 are isodipeptide cross-linked components of the human epidermal cornified cell envelope. J Biol Chem 270:17702–17711

Tabolacci C, Lentini A, Mattioli P, Provenzano B, Oliverio S, Carlomosti F, Beninati S (2010) Antitumor properties of aloe-emodin and induction of transglutaminase 2 activity in B16–F10 melanoma cells. Life Sci 87:316–324

Vermes I, Steur EN, Jirikowski GF, Haanen C (2004) Elevated concentration of cerebrospinal fluid tissue transglutaminase in Parkinson's disease indicating apoptosis. Mov Disord 19:1252–1254

Williams-Ashman HG, Canellakis ZN (1979) Polyamines as physiological substrates for transglutaminases. Perspect Biol Med 22:421–453

Wlodawer A, Mille M, Jaskolski M, Sathyanarayana BK, Baldwin E, Weber IT, Selk LM, Clawson L, Schneider J, Kent SBH (1989) Crystal structure of synthetic HIV-1 protease: conserved fold in retroviral proteases. Science 245:616–621

Yaffe MB, Murthy S, Eckert RL (1993) Evidence that involucrin is a covalently linked constituent of highly purified cultured keratinocyte cornified envelopes. J Invest Dermatol 100:3–9

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

Zainelli GM, Dudek NL, Ross CA, Kim SY, Muma NA (2005) Mutant huntingtin protein: a substrate for transglutaminase 1, 2, and 3. J Neuropathol Exp Neurol 64:58–65

Zemaitaitis MO, Lee JM, Troncoso JC, Muma NA (2000) Transglutaminase-induced cross-linking of tau proteins in progressive supranuclear palsy. J Neuropathol Exp Neurol 59:983–989

Zettergren JG, Peterson LL, Wuepper KD (1984) Keratolinin: the soluble substrate of epidermal transglutaminase from human and bovine tissue. Proc Natl Acad Sci USA 8:238–242

Zhang W, Johnson BR, Suri DE, Martinez J, Bjornsson TD (1998) Immunohistochemical demonstration of tissue transglutaminase in amyloid plaques. Acta Neuropathol 96:395–400