

## Review

# Transglutaminases in inflammation and fibrosis of the gastrointestinal tract and the liver

L. Elli<sup>a,\*</sup>, C.M. Bergamini<sup>b</sup>, M.T. Bardella<sup>a,c</sup>, D. Schuppan<sup>d</sup>

<sup>a</sup> Center for Prevention and Diagnosis of Celiac Disease, Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, via F. Sforza, Milan, Italy

<sup>b</sup> Department of Biochemistry, University of Ferrara, Via Luigi Borsari, Ferrara, Italy

<sup>c</sup> Department of Medical Sciences, University of Milan, via F. Sforza, Milan, Italy

<sup>d</sup> Beth Israel Deaconess Medical Center and Harvard Medical School, 330 Brookline Ave, MA Boston, USA

## ARTICLE INFO

## Article history:

Received 28 August 2008

Accepted 2 December 2008

Available online 4 February 2009

## Keywords:

Celiac disease

Collagen

Crohn's disease

Extracellular matrix

Gliadin

Inflammatory bowel disease

NFκB

Ulcerative colitis

## ABSTRACT

Transglutaminases are a family of eight currently known calcium-dependent enzymes that catalyze the cross-linking or deamidation of proteins. They are involved in important biological processes such as wound healing, tissue repair, fibrogenesis, apoptosis, inflammation and cell-cycle control. Therefore, they play important roles in the pathomechanisms of autoimmune, inflammatory and degenerative diseases, many of which affect the gastrointestinal system.

Transglutaminase 2 is prominent, since it is central to the pathogenesis of celiac disease, and modulates inflammation and fibrosis in inflammatory bowel and chronic liver diseases.

This review highlights our present understanding of transglutaminase function in gastrointestinal and liver diseases and therapeutic strategies that target transglutaminase activities.

© 2008 Editrice Gastroenterologica Italiana S.r.l. Published by Elsevier Ltd. All rights reserved.

## 1. Introduction

The major function of transglutaminases (TGs) is the covalent cross-linking of one protein chain via a glutamine residue to a lysine residue of another protein chain in a reaction termed transamidation. These enzymes are ubiquitously expressed in almost all mammalian cells and tissues, where they play important roles in the pathogenesis of various human disorders, including neurodegenerative and autoimmune diseases, inflammatory enteropathies, liver and pulmonary fibrosis and cancer [1]. These biological functions have been highlighted in reviews on wound healing and tissue repair [2], inflammation [3], apoptosis [4] and cancer progression [5]. Therefore, recent research has focused not only on the role of certain TGs in the pathomechanism of these diseases, but also on the therapeutic potential of their pharmacological inhibition [6]. Tissue TG or TG 2 (TG2) has attracted most interest, since it is found in almost all tissues, and is implicated in the pathogenesis of celiac disease (CD) [7], ulcerative colitis (UC), Crohn's disease (CsD) [8,9] and liver inflammation and fibrosis [10,11]. This review will summarize pertinent data on the role of TGs in inflammatory and

fibrotic diseases of the intestine and the liver and, discuss targeted therapeutic approaches.

## 2. Methods

The review is based on a comprehensive MEDLINE/Pubmed internet search (<http://www.ncbi.nlm.nih.gov/pubmed>) for articles published in English without any other limits using the MeSh terms: transglutaminase, transglutaminase 2, celiac disease, inflammatory bowel disease, Crohn's disease, liver, fibrosis, fibrogenesis, collagen, cross-link, isopeptide, apoptosis, phagocytosis, cirrhosis, ulcerative colitis, inflammation, cytokines.

## 3. Biochemistry of transglutaminases

### 3.1. Transglutaminases: What are they?

Mammalian tissues contain a large number of protein-processing enzymes; they include the proteinases (which degrade proteins during their usual turnover or modify them for regulatory purposes), and enzymes that promote aggregation and cross-linking of proteins, stabilizing tissues and cells against mechanical “wear-and-tear” and protect tissues against leakage of toxic materials from dying cells. Major enzymes which accomplish this

\* Corresponding author. Tel.: +30 02 55033384; fax: +30 02 50320403.

E-mail address: [lucelli@yahoo.com](mailto:lucelli@yahoo.com) (L. Elli).

fundamental function of tissue stabilization are the TGs, because they catalyse transamidation of specific glutamine residues of one protein chain either to the amino group of the side chain of a lysine residue in a second protein chain (resulting in the formation of a covalent N- $\gamma$ -glutamyl- $\epsilon$ -lysyl-isopeptide bond), or to the free amino group of a soluble amine such as a polyamine. An important feature of this cross-linking reaction is its virtual irreversibility, since the isopeptide bond is resistant to cleavage by proteinases. Therefore, the proteins cross-linked by TGs can effectively stabilize tissues [12] and can only be removed through their complete degradation [13].

### 3.2. Transglutaminases: How do they act?

Mammalian TGs activity is dependent on the presence of calcium. At increased calcium levels (above 0.5 mM) that are only achieved in extracellular fluids or in severely damaged or apoptotic cells, TGs form a covalent thioester intermediate with the distal free amide group of protein-bound glutamine residues via their active side thiol group. This initial step is accompanied by the release of one molecule of ammonia. The free thiol of the enzyme is regenerated by reaction with a second nucleophilic acceptor substrate (such as a lysine residue) which then reacts with the peptidylglutamyl-thioester intermediate to form the isopeptide bond (Fig. 1). In the absence of primary amines H<sub>2</sub>O can act as alternative acceptor through its negatively polarized oxygen atom, resulting in deamidation of the donor glutamine to a glutamic acid

residue. All these modifications change the properties of the substrate proteins, affecting their solubility (in case of cross-linking) or their charge, since an electrically neutral glutamine residue in the substrate protein is modified to a negatively charged residue (in case of hydrolysis to a glutamate residue) or positively charged in case of transamidation by the polyamines spermidine or spermine. Substrate specificity and the features of these reactions depend on the biochemical properties of the TGs, particularly their structure and regulation which have been reviewed recently [13,14].

This capacity of TG2 to modify proteins and their solubility contributes to the formation of dysfunctional aggregates of proteins with cytotoxic and proinflammatory capacity in neurodegenerative and autoimmune diseases [2]. For example, Huntington's, Alzheimer's and Parkinson's diseases are characterized by the deposition of abnormal proteins (huntingtin, extracellular  $\beta$ -amyloid and intracellular highly phosphorylated tau protein/neurofibrillary tangles/intracytoplasmic  $\alpha$ -synuclein aggregates, respectively), which are all substrates of TG2 [15].

### 3.3. Transglutaminases: How many are they and what are their functions?

TGs are a family of enzymes with at least 8 isoforms, products of different genes (TG1 to TG7 and plasma Factor XIII), along with possible splicing variants which are described with increasing frequency and which often display altered catalytic properties. FXIII is involved in blood clotting and wound healing, TG1 and TG3 in epidermal terminal differentiation and TG4 in reproduction, while the functions of TG5, TG6 and TG7 are still unclear. The structural organization of TGs is discussed extensively in ref. [12]. Many studies focused on TG2 which was described as the "bête noire" of the TGs family [16] because it is a pleiotropic protein with numerous functions detailed below. However, TG3 has gained relevance in GI pathophysiology also. Expression of TG3, which is produced as a zymogen requiring proteolytic cleavage at an internal region between domains 2 and 3 for activity, is linked to epidermal differentiation. Its relevance for CD is restricted to those patients presenting with dermatitis herpetiformis (DH) which is accompanied by an autoimmune reaction against this TG isoform [17].

Apart from transamidation or deamidation activities, TG2 which is the best studied and probably the most relevant isoform, displays additional activities as a G-protein, protein disulfide isomerase and a protein kinase. In addition, it tightly associates non-covalently and covalently with certain integrins (major receptors of the extracellular matrix–ECM) and with fibronectin and several other ECM proteins. Through the selective deployment of some of these activities, TG2 plays multiple roles in cell physiology, such as the regulation of apoptosis (through its intracellular cross-linking activity), signal transduction, inflammation and regulation of cell-cycle progression (by acting as a G-protein). While high calcium concentration and the availability of acceptor ligand trigger activation of TG2, enzymatic cross-linking activity is inhibited by guanosine triphosphate (GTP), which on the other hand switches-on signal transduction. This occurs at physiological concentrations of GTP, while in pathological situations both the decline in cellular GTP (with increase in GDP), the increase in guanosine diphosphate (GDP), and the intracellular influx of calcium promote the cross-linking function of TG2 [18]. It was postulated that the TG2-GDP complex is inactive when also associated with calreticulin [19], while it gets activated by interaction with the  $\alpha$ 1B-adrenergic [20], oxytocin [21] and thromboxane A2 receptors [22] which results in an exchange of GDP for GTP and dissociation of the TG2-GTP/calreticulin complex. TG2-GTP then activates the downstream effector phospholipase C $\delta$ 1 (PLC $\delta$ 1) generating phosphatidylinositol-bisphosphate that triggers release of calcium from intracellular stores. Moreover, PLC $\delta$ 1 is able to promote and

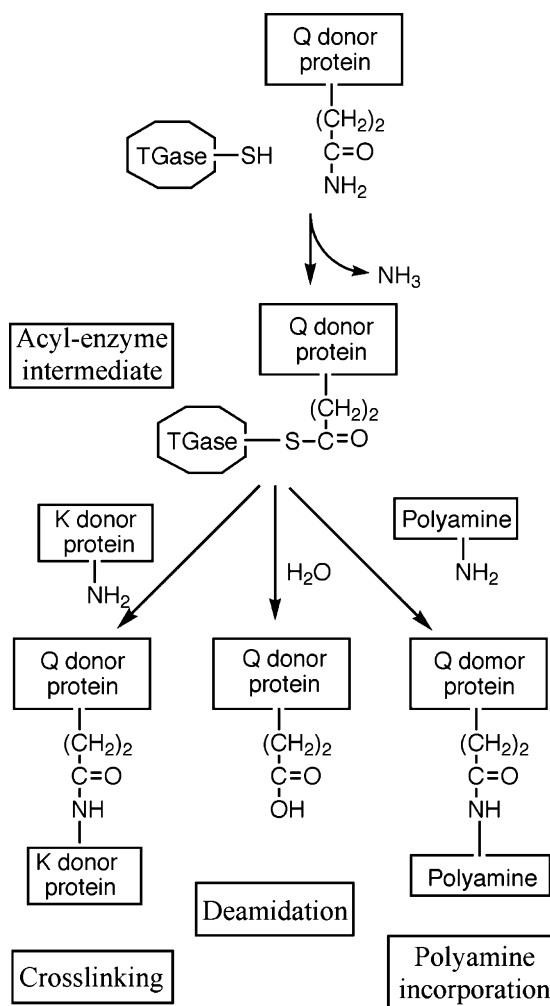


Fig. 1. Biochemistry of transglutaminase 2.

stabilize the TG2-GTP interaction, acting as guanine nucleotide exchange factor and a GTPase inhibiting factor. The whole process is switched-off by GTP hydrolysis and the reconstitution of the TG2-GDP/calreticulin complex [18].

A fraction of TG2 that is anchored as an active ecto-enzyme at the external surface of cell membranes or that is associated with fibronectin modulates cell adhesion and organization of the ECM through its interaction with integrins and complex formation with and cross-linking of ECM proteins, such as fibronectin (FN) stabilizing the integrin-ECM (FN) interaction [23]. Integrins are heterodimers of an  $\alpha$  and a  $\beta$  subunit many of which (e.g.,  $\alpha 5\beta 1$ ,  $\alpha V\beta 3$ ,  $\alpha V\beta 5$ ,  $\alpha V\beta 6$ ,  $\alpha V\beta 8$ ,  $\alpha 8\beta 1$  and  $\alpha IIb\beta 3$ ) recognize the RGD (Arg-Gly-Asp) motive in their ECM ligands, including FN. As  $\beta$  integrin co-receptor TG2 is also able to transmit signals from the extracellular to the intracellular environment through the activation of the RhoA/ROCK and the focal adhesion kinase signalling pathways [24]. Thus, the diverse functional features of TG2 are related to the stabilization of different conformations by the small molecular modulators such as calcium and GTP, by multiple interactions with the ECM and integrins [13], and in part autocatalytical cross-linking (and inactivation) by reaction with its peptide substrates when TG2 serves as its own glutamine donor [3].

#### 4. Pathophysiology of transglutaminase 2

##### 4.1. Activators of transglutaminase 2 transcription

The TG2 gene is located on chromosome 20q11-12 and is composed of 13 exons and 12 introns [25]. The 5' terminal region contains different regulatory sequences which can activate the transcription of the TG2 gene by the interaction with various transcription factors. Among the most studied TG2 inducers are the retinoic acid derivatives (especially all trans retinoic acid, ATRA) which, via the retinoid acid receptor alpha [26], activate TG2 transcription through interaction with a retinoic acid response element located 1.7 kb upstream of the translation initiation site. The induction of TG2 by ATRA is important in cell differentiation, cell survival or apoptosis, depending on the cellular context [27]. Thus, through the activation of TG2, ATRA triggered neuronal differentiation and cell survival in a neuroblastoma cell line [27], while it promoted apoptosis in pancreatic adenocarcinoma cells [28]. Similarly, multiple other mediators induce TG2 transcription, such as cyclic AMP [29], dexamethasone [30], sodium butyrate [31], phorbol esters [32], hydrogen peroxide [33], Vitamin D [34] and statins [35]. In addition, cytokines and growth factors regulate TG2 transcription. Transforming Growth Factor  $\beta$  (TGF $\beta$ ) enhances TG2 gene expression through a response element located 868 bases upstream of translation initiation, which is part of a positive feedback loop, since TG2 binds latent TGF $\beta$ 1 to the cell surface, where it becomes proteolytically processed to biologically active TGF $\beta$ 1, thus acting as a co-activator of TGF $\beta$ 1 [36]. Epidermal growth factor (EGF) [37], interleukin-6 (IL6) [38], interferon  $\gamma$  and  $\beta$  (IFN $\gamma$  and IFN $\beta$ ) [39,40] and tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) [41] also induce transcriptional activation of TG2.

##### 4.2. The role of transglutaminase 2 in TGF $\beta$ 1 activation and implications for cellular differentiation

Differentiation, remodeling and homeostasis of the intestinal epithelium are strictly controlled to avoid abnormal cell growth or mucosal atrophy in view of a high cell turnover. In the gut, epithelial growth control is maintained largely through the crypt compartment and migration of the crypt cells to the tip of the villus, which is accompanied by their differentiation and by a regulated apoptosis. During their passage from crypt to villus along the crypt-villus axis, enterocytes alter their gene expression which is controlled

by reciprocal mesenchymal-epithelial interactions and, above all, by growth factors secreted by the pericryptal fibroblasts [42]. One of the most important modulator of enterocyte differentiation is TGF $\beta$ 1, a multifunctional cytokine which, via direct effects on the cell cycle, steers cellular growth and differentiation, but depending on local concentrations and cell type also induces apoptosis. Furthermore, TGF $\beta$ 1 is the strongest known promoter of wound healing and fibrosis, a potent inhibitor of inflammation [43,44] and an inducer of epithelial-mesenchymal differentiation [45].

The tight association of TG2 with TGF $\beta$ 1 in a positive feedback loop, implicates TG2 in the control of cell survival, cell cycle and cellular differentiation. This is supported by the findings that TG2 KO mice present decreased levels of TGF $\beta$ 1 (as well as a deficiency of macrophage phagocytotic activity [46]), and that inhibition of TG2 by function blocking antibodies blunted the differentiation state of T84 intestinal cells in an in vitro coculture model with fibroblasts [44]. However, TGF $\beta$ 1 suppressed TG2 mRNA in IEC-6 rat small intestinal epithelial cells [39], and neutralizing anti-TGF $\beta$  antibodies reduced the expression of TG2 in mice [46], suggesting that the interaction between TG2 and TGF $\beta$ 1 is context-dependent and more complex.

##### 4.3. The role of transglutaminase 2 in apoptosis

Programmed cell death is a central process for maintaining normal growth, differentiation and tissue homeostasis. Increased apoptosis of epithelial cells is found in CD [47,48] and inflammatory bowel disease (IBD) [49]. The final events of programmed cell death lead to activation of proteolysis and protein packaging. The latter is dependent on TG2-mediated cross-linking, preventing leakage of intracellular proteins into the extracellular space, favouring phagocytosis by macrophages and protecting the body from autoimmunity [50,51]. This intracellular cross-linking activity of TG2 occurs via an increase in calcium influx when cells are subject to extreme stress, such as during chemotherapy, hypoxia or absence of essential growth factors.

In this area, several studies demonstrated the proapoptotic role of TG2 in vitro in different cell types (SK-N-BE neuroblastoma cells, Balb-C 3T3 fibroblasts, Human embryonic kidney 293 cells and promonocytic U937 cells) [52–54]. Furthermore, TG2 KO mice have defects in the clearance of dying cells, while TG2 transfected HEK 293 cells undergo apoptosis [46,53]. The finding that TG2 may also be antiapoptotic in several cell types (SH-SY5Y neuroblastoma cells, SVC1 and Ki-SVC1 epithelial cells, HL60 leukaemia cells) [4,55–57] can be explained by the cellular compartmentalization of the enzyme. TG2 is localized on the outer cell membrane, in the cytosol and inside the nucleus, and only the cytosolic form can drive apoptosis, whereas both the cell membrane and the nuclear form have an antiapoptotic function [53]. Thus under conditions of cellular stress, cytosolic TG2 oligomerizes death-associated protein (DAP)-like kinase (DLK), a nuclear serine/threonine-kinase implicated in apoptosis through the activation of c-Jun N-terminal kinase (JNK) (mainly JNK-1 but data are not definitive), a key stress response kinase and regulator of apoptosis [58,59]. Within the nucleus, through its interaction with importin- $\alpha 3$  [60], TG2 interacts with the retinoblastoma protein (Rb) and enhances its function as apoptosis suppressor [61]. Here TG2 maintains Rb in a phosphorylated (antiapoptotic) state in environmental conditions which suppresses its cross-linking activity (high levels of guanosine or adenosine nucleotides, low calcium concentration) [62,63]. A role of the Rb-TG2 interaction in the suppression of apoptosis is also suggested by the inhibition of caspase-mediated degradation of Rb after exposure of cells to ATRA, a well-known TG2 inducer and the loss of the antiapoptotic activity of ATRA in Rb KO mice [62]. Antiapoptotic activity of ATRA has been proposed to be mediated by active phosphoinositide 3-kinase (PI3K), which regulates the interaction

of TG2 with GTP [64]. How PI3K promotes the binding of GTP to TG2 is still unknown, but PI3K may regulate the expression/activation of a hypothetical guanine nucleotide exchange factor for TG2, similarly to the mechanism implicated in the activation of other small G proteins [65]. Finally, the ability of TG2 to interact with  $\beta$  integrins and stabilize the contact between the cells and the ECM represents in itself a survival promoting and antiapoptotic function, which has been implicated in chemoresistance of cancers [66]. Taken together, the cross-linking activity in the cytosol favours apoptosis, while the GTP-form of TG2, its involvement in the stabilization of ECM contacts and its translocation into the nucleus to interact with Rb or other nuclear proteins are antiapoptotic.

#### 4.4. The role of transglutaminase 2 in inflammation

TG2 can act as a modulator of inflammation, exerting both pro- and anti-inflammatory effects such as in the previously described positive feedback loop with TGF $\beta$ 1 [67]. TG2 enhances the activity of the secretory isoform of phospholipase A2 (sPLA2) through the formation of an intramolecular isopeptide bond within the enzyme and therefore increases the biosynthesis of proinflammatory eicosanoids [2]. Nonapeptides that are derived from the TG2 target sites in uteroglobin and lipocortin-1 block this TG2-mediated PLA2 activation and have a strong anti-inflammatory effect in vivo [68]. NF- $\kappa$ B, a central downstream mediator of inflammation in IBD [69], exerts a proinflammatory effect in immune cells and a predominant anti-inflammatory role in colonic epithelial cells [70]. NF- $\kappa$ B is activated by TG2 which polymerizes and therefore inactivates its inhibitor, I $\kappa$ B $\alpha$ , by cross-linking its C-terminal glutamine cluster [71]. Recent data demonstrated that TG2 KO mice are more resistant to septic shock than wild-type mice, with mitigated multiorgan injury, mainly linked to a reduction of NF $\kappa$ B nuclear translocation [72]. TG2 can also stimulate inflammation through the aggregation and functional sequestration of the anti-

inflammatory factor peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ), as demonstrated in a model of cystic fibrosis, where the specific in vitro inhibition of TG2 is able to reinstate PPAR $\gamma$  and inflammatory cytokine levels [73].

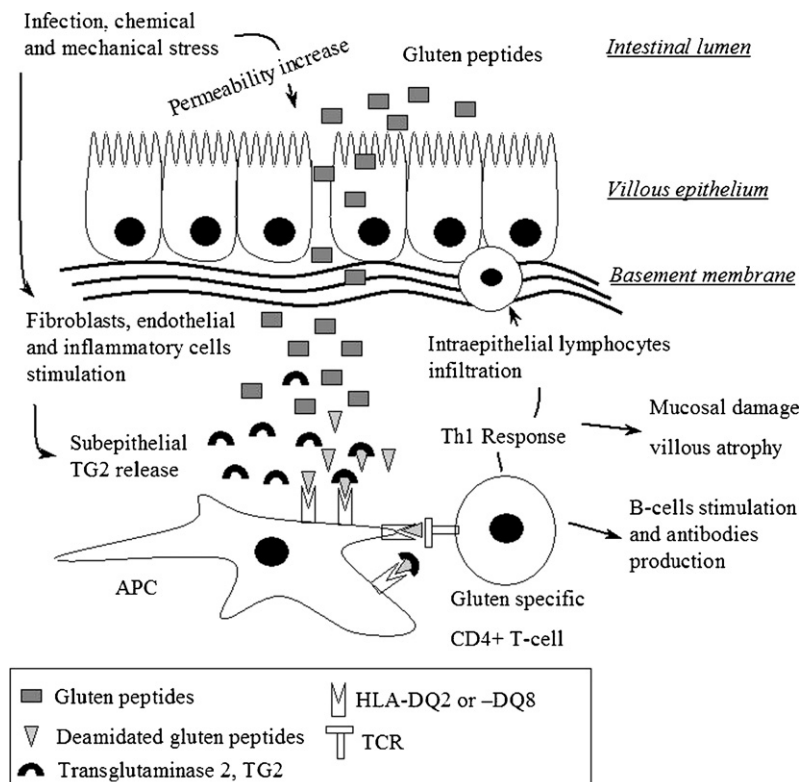
Moreover, after the discovery that TG2 acts as an integrin co-receptor, it was demonstrated that inhibition of this cell surface-bound TG2 with a monoclonal antibody can block transendothelial migration of human CD8 $^{+}$  T cells in vitro [74].

### 5. Transglutaminase 2 in gastrointestinal diseases and liver fibrosis

#### 5.1. Transglutaminases and celiac disease

CD is a common enteropathy characterized by an altered immune-response to dietary wheat gluten and related proteins from rye and barley. Celiac disease is triggered and maintained, in genetically predisposed patients carrying human lymphocyte antigen (HLA)-DQ2 or -DQ8, by both an innate and an acquired (adaptive, largely a T helper type 1 (Th1) T cell phenotype) immune response to gluten [75].

CD diagnosis is based on proximal small bowel biopsy findings characterized by villous atrophy, crypt hyperplasia and increased numbers of intraepithelial lymphocytes [76]. Clinical suspicion in subjects on a gluten containing diet was supported by the presence of serum IgA autoantibodies against the connective tissue that surrounds smooth muscle fibres of the esophagus (antiendomysium antibodies—EMA) [77]. The target autoantigen was later identified as TG2 [78], a finding that spawned numerous studies aimed to (a) elucidate the role of TG2 in the pathogenesis of CD and other autoimmune diseases, (b) examine its role in the regulation of growth factors, inflammation, wound healing and repair and (c) clinically validate assays measuring TG2 autoantibodies for the diagnosis of CD and for population screening.



**Fig. 2.** Involvement of transglutaminase 2 in the pathogenesis of celiac disease. Transglutaminase 2 (TG2); antigen presenting cell (APC); human leukocyte antigen (HLA); T-cell receptor (TCR).



## 5.2. The role of transglutaminases in the pathogenesis of celiac disease

CD is defined HLA-linked disorder, since: (a) the trigger is well defined (certain gluten peptides), (b) HLA-DQ2 or -DQ8 is the essential genetic predisposition and (c) it is uniquely associated with a mucosal (IgA) autoimmune response to TG2 [75]. TG2 is an enzyme that can either cross-link or deamidate gluten, and the deamidated peptides bind more strongly to DQ2 or DQ8 which results in Th1 T cell activation and mucosal destruction. Due to their high glutamine content (>30%) and their peculiar structure with a high proportion of proline residues, gliadins and glutenins (the storage proteins that constitute gluten) are very good substrates for TG2. It is primarily the frequently occurring sequence motive Proline-Glutamine-X (various amino acid residues)-Proline-Tyrosine (or Phenylalanine), in which the glutamine residue has been deamidated by TG2 to glutamic acid, that binds with higher affinity to HLA-DQ2 (HLA-DQ8) on antigen presenting cells in the intestinal lamina propria, resulting in a strong gluten specific Th1 T cell response [79,80] (Fig. 2).

The production of autoantibodies to TG2 appears to be fueled by TG2 that has covalently linked itself (as lysine substrate) to gliadin (glutenin) peptides in a process of autocatalysis [81]. This is supported by the detection of conjugates of TG2 with gliadin peptides bound either at the active site of the enzyme via a thioester bond or at various lysine residues via the isopeptide linkage characteristic of transglutaminase-catalyzed protein cross-links [82]. Peptides bound via the thioester linkage stabilize the usually transient open conformation of TG2, and thus trigger antibody production, in part to normally cryptic epitopes [83]. The prevailing opinion is that the produced autoantibodies can block up to 90% of TG2 activity in vitro [84], but is unclear how far this affects TG2 function in vivo. In a coculture model using colon T84 epithelial cells and fibroblasts, patients' autoantibodies prevented TGF $\beta$ 1 activation (in which cell surface TG2 is involved) and consequently epithelial differentiation [44], suggesting that local autoantibody production may at least inhibit partial functions of the enzyme.

DH is a blistering skin disease that is characterized by deposition of IgA antibodies around vessel walls and at the dermoepidermal junction in response to gluten. The immune complexes are composed of IgA autoantibodies and epidermal TG (TG3) as autoantigen [17]. No other TG is present in these complexes, but the development of DH is clearly dependent on oral gluten exposure, since patients carry HLA-DQ2 or -DQ8 and usually have mild intestinal lesions characteristic of CD. Interestingly, the autoantibodies of patients with dermatitis herpetiformis that are directed to TG3 cross-react with TG2, and patients have both circulating autoantibodies to TG2 and TG3 [85]. The main roles played by TG2 in CD are summarized in Fig. 3.

## 5.3. The role of transglutaminase 2 in the regulation of intestinal growth factors, inflammation, wound healing and repair

Activated TG2 in the extracellular compartment of the epithelial layer and the lamina propria of the intestine serves as a bait for

gluten and favours the antigenic presentation of gluten peptides to lymphocytes, followed by a local Th1 T cell activation and subsequent production of IFN $\gamma$ . IFN $\gamma$  is itself a positive regulator of TG2 gene transcription, in concert with TNF $\alpha$  [2]. TG2 can also cross-link gliadin peptides to several collagen types causing their immobilization within the ECM of the lamina propria (there availability to the immune system is protracted) and resulting in elevated circulating collagen autoantibodies [86]. TG2 expression is also regulated by polyamines, which are natural TG2 substrates and are necessary for the intestinal tissue repair mechanism [87]. Although not conclusive, cell culture studies have shown that polyamines act through different pathways, transcriptionally regulating the expression of the TG2 gene, and post-transcriptionally influencing TG2 mRNA half life [88,89].

Therefore, in genetically predisposed subjects, elevated levels of active extracellular TG2 can be induced by cellular stressors, especially (unspecific) inflammation and consequent cytokine release, facilitating gliadin deamidation. This includes bacterial lipopolysaccharide [90], which elicits synthesis of cytokines, mainly IL6 and TNF $\alpha$  [3], the latter being involved in a positive feedback loop with TG2 as previously mentioned [71]. Moreover, infection with different viruses (HIV, HPV or HCV) induces an increase of TG2 with consequent apoptosis and viral protein modifications via cross-linking [91]. It is possible that benign enteric infections, such as oxidative or mechanical stressors that induce the secretion of TG2 [92], trigger CD in genetically predisposed subjects. The key role that TG2 plays in CD pathogenesis has lead to the development of small molecule TG inhibitors that could be used in the treatment of CD as an alternative therapy [6,93] (see below).

## 5.4. Clinical validation of assays for transglutaminase 2 autoantibodies in the diagnosis of celiac disease

Solid-phase ELISAs based on recombinant human TG2 are highly sensitive and specific tools for the measurement of increased IgA autoantibody titres in the diagnosis of untreated patients with CD, with reported sensitivities and specificities above 97% and 98% [94,95] for current commercial assays and depending on the population screened [96]. This blood test is now recommended as first non-invasive tool to diagnose CD. However, in patients with CD who are on a gluten-free diet there is not a good correlation between negative titres and (complete) normalization of the intestinal mucosa [97], but anti-TG2 antibodies remain useful to detect patients with low dietary compliance [77,98,99]. Recently, a new assay based on a set of gliadin peptides that are deamidated at TG2 target sites has been developed [100,101]. Anti-immunoglobulin A and G levels to these deamidated peptides predict CD with a similar accuracy as anti-TG2 antibodies, and the combination of both tests appears to further increase sensitivity without lowering specificity.

## 5.5. Transglutaminases and inflammatory bowel disease

IBD is a spectrum of T cell mediated chronic intestinal disorders grossly divided into two major forms: UC and CsD. Although the pathogenesis of IBD is still unknown, numerous studies showed that both UC and CsD are driven by an abnormal response of the enteric immune system towards the luminal flora. Patients with CsD mainly develop an intestinal immune response dominated by a Th1 phenotype and prominent production of IFN $\gamma$  and IL2, while patients with UC display predominantly a CD4+ Th2 phenotype with production of IL5 and IL6. In addition, other ILs (IL1 and TNF $\alpha$ ), chemokines and inflammatory mediators, such as arachidonic acid metabolites and reactive oxygen species are involved in the inflammatory processes of both diseases. Moreover, recent studies suggest the involvement,

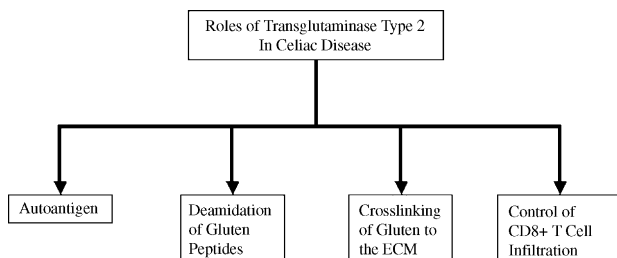


Fig. 3. Main roles of transglutaminase 2 in celiac disease.

in the pathogenesis of IBD, of the CD 4+ Th17 phenotype, that links innate and adaptive immunity [102,103].

Current IBD therapies are based on anti-inflammatory or immunosuppressive drugs showing little selectivity, potential side effects and are ineffective in some patients. Therefore, current efforts are focused to discover novel pathways and factors that are involved in the pathogenesis of IBD to develop better targeted agents [103].

TG activity is upregulated in the intestinal mucosa, especially in the lamina propria of patients with IBD [104], but plasma TG activity (which is due to factor XIII) is inversely correlated with the activity and severity of the disease [8,104,105]. In patients with active UC, both TG2 and FXIII were increased in the colonic mucosa, mainly in damaged or apoptotic colonocytes, basement membranes and the ECM, in colocalization with isopeptide bonds, indicating transamidating activity [9]. Of note, not all TGs followed the same trend, since colonic expression of TG1 was downregulated [9]. In the skin, TG1 is expressed during epidermal differentiation and the establishment of the epidermal barrier, suggesting that its downregulation heralds loss of the intestinal barrier function in UC [106].

Based on these data and on the efficiency of intravenous administration of FXIII in the rat model of colitis induced by trinitrobenzene sulfonic acid [107], supplementation with FXIII has been suggested as a treatment of IBD. However, clinical studies in patients with IBD remained inconclusive [108,109], and the only large randomized controlled trial that enrolled steroid refractory patients was negative [110].

Since TG2 was implicated in the pathogenesis of IBD, different studies investigated the presence of anti-TG2 antibodies (IgA or IgG) in IBD patients and a possible association with CD. A study of 305 patients with CD showed a tenfold higher autoantibody prevalence in IBD (including indeterminate and microscopic colitis) compared to the control population [111], while the prevalence of CD in 354 patients with IBD was similar to that of the controls, with most IBD patients having false positive anti-TG2 IgA antibodies. However, the fraction (up to 10%) of autoantibody positive patients with IBD (CsD>UC) in whom CD was excluded by negative duodenal biopsies, only displayed low or borderline titres [112–114]. These low titre autoantibodies to TG2 may be due to antigenic epitope spreading that could occur during chronic inflammation of the intestinal mucosa. In this vein, a study found low titre autoantibodies to TG2 in 1.9% of patients with connective tissue diseases, such as systemic lupus erythematosus, rheumatoid arthritis, systemic sclerosis, Sjogren's syndrome, UC and primary biliary cirrhosis [115], while only 0.3% of these patients had histologically proven CD, a

percentage lower than that expected in general population (0.5–1%) [116–119].

A causative role of TG2 in the specific immuno-pathogenesis of these diseases, as in CD, is unlikely, since these antibodies are only found in a minority of the patients. Nonetheless, it is highly likely that TGs (and TG activity in general) play an important role in the pathogenesis of IBD, but mechanistic studies are missing, especially to clarify if inhibition or activation of enzymatic activity would be beneficial. Thus, TG2 and TG activity in general has been linked both to pro- and anti-inflammatory, and to pro- and anti-apoptotic effects. The finding of decreased serological TG activity and increased TG2/FXIII mucosal expression, together with the demonstration of an improvement of colitis by the inhibition of calpain (this proteinase is involved in proteolytic degradation of TG2 in tissues and its inhibition increases TG2 protein and activity *in situ*), supports the hypothesis that restoration of TG activity could be beneficial for intestinal wound healing in IBD [120,121]. As TG2 is a key enzyme in wound healing and tissue repair that confines cell necrosis, stabilizes the ECM, and seals epithelial tight junctions, a targeted repletion or activation of TGs in the gut should be a potential therapy for IBD. The reported inflammatory roles TGs in IBD are summarized in Fig. 4.

#### 5.6. Transglutaminase 2 in liver disease

When exposed to repetitive damage (viral, toxic, metabolic) the liver usually reacts with a chronic wound healing response that usually leads to fibrosis, i.e., excess accumulation of ECM. When fibrogenesis (de novo synthesis and deposition of ECM) continues to outbalance fibrolysis (removal of ECM), cirrhosis, i.e., advanced fibrosis with severe distortion of the liver vascular architecture, ensues. Cirrhosis is the most relevant predictor of morbidity and mortality in patients with chronic liver disease, and major efforts are currently focused on the development of antifibrotic agents that halt fibrosis progression or even induce its reversal [122,123].

The ECM is a complex structure composed of collagens, proteoglycans, glycosaminoglycans, noncollagenous (glyco-) proteins, ECM-bound growth factors, proteases and enzymes, that provide anchoring, structure and signals to the cells embedded in it. Vice versa the cells condition their ECM. In the normal liver, a low density ECM is present in the space of Disse separating hepatocytes from the sinusoidal endothelium, whereas high density ECM is localized in the portal area [124]. Liver fibrogenesis is mainly driven by activated hepatic stellate cells and perivascular or portal fibroblasts that transform to excess ECM producing myofibroblasts (MFs), cells

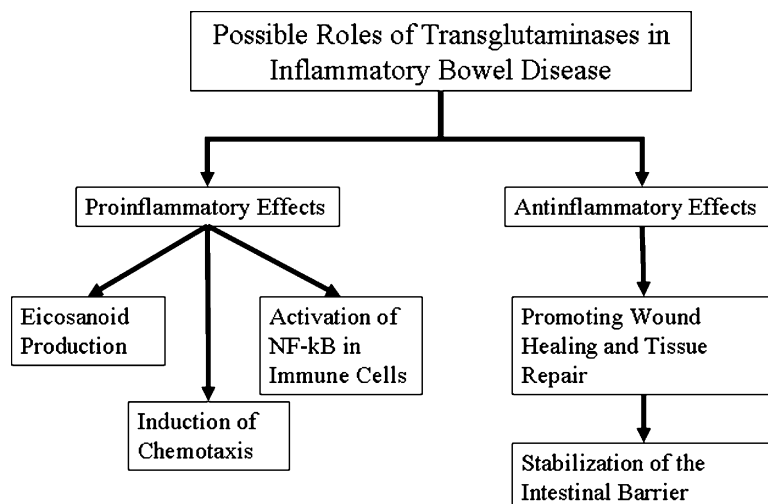


Fig. 4. Possible roles of transglutaminases in inflammatory bowel disease.

that resemble, e.g., dermal or intestinal myofibroblasts in scar or stricture formation, respectively. Since a variety of ECM molecules (such as procollagens, fibronectin or laminins) are TG2 substrates, and since important regulators of wound healing and inducers of fibrogenesis (TGF $\beta$ , TNF $\alpha$ , IL6) promote TG2 expression, TG2 has been implicated in liver fibrogenesis [125,126]. Thus TG2 has been considered profibrogenic, since its cross-linking activity stabilizes the ECM network, conferring resistance to proteolytic breakdown. This was demonstrated in fibrotic liver specimens from patients with chronic hepatitis B or C and with alcoholic hepatitis, where high levels of TG2 are detected extracellularly and in association with the formation of N- $\gamma$ -glutamyl- $\epsilon$ -lysyl cross-links as a measure of TG2 activation [127]. In this line, TG2 expression was also correlated with fibrosis stage in biopsies of patients with chronic hepatitis C in one study [128].

TG2 may facilitate hepatic fibrogenesis also through its role in cell surface activation of TGF $\beta$ 1, the most potent profibrogenic cytokine. On the other hand, the effect of the TG2-mediated increase in NF $\kappa$ B signalling, e.g., in response to TNF $\alpha$  is far less clear, since TNF $\alpha$  may exert fibrolytic as well as fibrogenic activities. An in vivo study found that spontaneous reversal of CCl $_4$ -induced micronodular cirrhosis over one year only proceeded to the stage of macronodular cirrhosis, and that further reversal was apparently limited by collagen cross-linking, due at least in part to TG-mediated cross-links [129]. In contrast to this supposedly profibrogenic activity of TG, the same group reported that a TG2-cross-linked collagen matrix inhibited hepatic stellate proliferation and collagen I synthesis in vitro [130]. On the other hand, TG2 knockout mice with CCl $_4$ -intoxication display high lethality as compared to their wild-type controls [11]. TG2 is a key enzyme that promotes Mallory bodies formation in alcoholic and non-alcoholic steatohepatitis via cross-linking and aggregation of cytokeratins [10]. Therefore, enhanced TG2 enzymatic activity seems to protect the liver from acute and chronic injury, while its net effect on fibrogenesis needs further study, e.g., by use of specific (TG2) inhibitors.

## 6. Inhibitors and activators of transglutaminase: current knowledge and future perspectives

Since TGs share a high degree of sequence similarity, especially in their catalytic centre [131], current inhibitors do not display selectivity, e.g., for TG2. Inhibitors that target TG cross-linking activity have been developed and mainly tested in vitro [6,132–136]. These inhibitors can be divided into three main groups: (a) competitive inhibitors (putrescine, spermidine, histamine, monodansyl cadaverine, cadaverine, 5-pentylamine, fluorescein, cystamine and cysteamine) [137], (b) reversible allosteric inhibitors (the GTP analogues, guanylyl  $\beta$ , $\gamma$ -methylene-diphosphonate, Mg-GTP, GTP $\gamma$ S, Zn $^{2+}$  and a class of molecules with thieno-[2,3-d]pyrimidin-4-one acylhydrazide backbones) [138–141] and (c) irreversible inhibitors (iodoacetamide, 3-halo-4,5-dihydroisoxazoles, carbobenzyloxy-L-glutamylglycine derivatives, 6-diazo-5-oxo-norleucine, 2-[(2-oxopropyl)thio]imidazolium derivatives) [142–146]. In particular, cysteamine acts not only as a competitive amine substrate that inhibits TG2 cross-linking activity, but also as an inhibitor through the formation of a disulfide bond with the TG2 active site [147]. Cystamine and the 2-[(2-oxopropyl)thio]imidazolium inhibitors (L682777 or R283) have been tested *ex vivo* in cultures of small intestinal biopsies of CD patients, where they blunted T cell stimulatory activity of gliadin peptides [148,149]. The administration of an irreversible TG2 inhibitor (halo-dihydroisoxazole derivative KCC009) did not prevent the formation of Mallory bodies in mice fed with 0.1% 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) as a model of liver injury, but prevented hepatomegaly [150,151]. Moreover, cystamine ameliorated liver fibrosis and reduced transaminases in rats after intraperitoneal injections of

CCl $_4$  [152]. However, these results that contradict the above mentioned studies in wild-type vs. TG2 KO mice must be interpreted with care, since cystamine or cysteamine also inhibit caspase-3 [153] and increase the cellular glutathione content [154,155].

Other molecules have been shown to increase TG2 activity. As mentioned before, ATRA increases TG2 expression and is widely available [156]. Interestingly, recent data showed that ATRA mediates homing of dendritic and T cells to the gut [157] and that the induction of TGF $\beta$ 1 by ATRA supports the conversion of naive T cells into regulatory T cells, diverting the Th17-promoting effect of IL6 [158]. In theory, the known strong connection between TGF $\beta$ 1 and TG2 suggests a potential role of TG2 and TG2 inhibition in intestinal immune modulation.

Essentially nothing is known about the effects of inhibition of the non-enzymatic functions of TGs, such as the G protein function of TG2. Taken together, more in vivo studies with well defined and, if possible, TG subtype specific inhibitors are necessary to fathom TG function in gastrointestinal and liver diseases, and to fully assess the therapeutic potential of pharmacological TG modulation.

## Conflict of interest statement

None declared.

## References

- [1] Lorand L. Crosslinks in blood: transglutaminase and beyond. *Faseb J* 2007;21:1627–32.
- [2] Verderio EA, Johnson TS, Griffin M. Transglutaminases in wound healing and inflammation. *Prog Exp Tumor Res* 2005;38:89–114.
- [3] Kim SY. Transglutaminase 2 in inflammation. *Front Biosci* 2006;11:3026–35.
- [4] Fesus L, Szondy Z. Transglutaminase 2 in the balance of cell death and survival. *FEBS Lett* 2005;579:3297–302.
- [5] Mangala LS, Mehta K. Tissue transglutaminase (TG2) in cancer biology. *Prog Exp Tumor Res* 2005;38:125–38.
- [6] Siegel M, Khosla C. Transglutaminase 2 inhibitors and their therapeutic role in disease states. *Pharmacol Ther* 2007;115:232–45.
- [7] Dieterich W, Esslinger B, Schuppan D. Pathomechanisms in celiac disease. *Int Arch Allergy Immunol* 2003;132:98–108.
- [8] D'Argenio G, Biancone L, Cosenza V, Della Valle N, D'Armiento FP, Boirivant M, et al. Transglutaminases in Crohn's disease. *Gut* 1995;37:690–5.
- [9] D'Argenio G, Calvani M, Della Valle N, Cosenza V, Di Matteo G, Giorgio P, et al. Differential expression of multiple transglutaminases in human colon: impaired keratinocyte transglutaminase expression in ulcerative colitis. *Gut* 2005;54:496–502.
- [10] Strnad P, Harada M, Siegel M, Terkeltaub RA, Graham RM, Khosla C, et al. Transglutaminase 2 regulates mallory body inclusion formation and injury-associated liver enlargement. *Gastroenterology* 2007;132:1515–26.
- [11] Nardacci R, Lo Iacono O, Ciccosanti F, Falasca L, Addesso M, Amendola A, et al. Transglutaminase type II plays a protective role in hepatic injury. *Am J Pathol* 2003;162:1293–303.
- [12] Greenberg CS, Birckbichler PJ, Rice RH. Transglutaminases: multifunctional cross-linking enzymes that stabilize tissues. *Faseb J* 1991;5:3071–7.
- [13] Griffin M, Casadio R, Bergamini CM. Transglutaminases: nature's biological glues. *Biochem J* 2002;368:377–96.
- [14] Esposito C, Caputo I. Mammalian transglutaminases. Identification of substrates as a key to physiological function and physiopathological relevance. *FEBS J* 2005;272:615–31.
- [15] Ruan Q, Johnson GV. Transglutaminase 2 in neurodegenerative disorders. *Front Biosci* 2007;12:891–904.
- [16] Lorand L, Graham RM. Transglutaminases: crosslinking enzymes with pleiotropic functions. *Nat Rev Mol Cell Biol* 2003;4:140–56.
- [17] Cannistraci C, Lesnori La Parola I, Cardinali G, Bolasco G, Aspate N, Stigliano V, et al. Co-localization of IgA and TG3 on healthy skin of coeliac patients. *J Eur Acad Dermatol Venereol* 2007;21:509–14.
- [18] Mhaouty-Kodja S. Ghalpha/tissue transglutaminase 2: an emerging G protein in signal transduction. *Biol Cell* 2004;96:363–7.
- [19] Feng JF, Readon M, Yadav SP, Im MJ. Calreticulin down-regulates both GTP binding and transglutaminase activities of transglutaminase II. *Biochemistry* 1999;38:10743–9.
- [20] Nakaoka H, Perez DM, Baek KJ, Das T, Husain A, Misono K, et al. Gh: a GTP-binding protein with transglutaminase activity and receptor signaling function. *Science* 1994;264:1593–6.
- [21] Park ES, Won JH, Han KJ, Suh PG, Ryu SH, Lee HS, et al. Phospholipase C-delta1 and oxytocin receptor signalling: evidence of its role as an effector. *Biochem J* 1998;331(Pt 1):283–9.
- [22] Vezza R, Habib A, FitzGerald GA. Differential signaling by the thromboxane receptor isoforms via the novel GTP-binding protein. *Gh J Biol Chem* 1999;274:12774–9.

- [23] Zemskov EA, Janiak A, Hang J, Waghray A, Belkin AM. The role of tissue transglutaminase in cell-matrix interactions. *Front Biosci* 2006;11:1057–76.
- [24] Janiak A, Zemskov EA, Belkin AM. Cell surface transglutaminase promotes RhoA activation via integrin clustering and suppression of the Src-p190RhoGAP signaling pathway. *Mol Biol Cell* 2006;17:1606–19.
- [25] 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;276:33066–78.
- [26] Giandomenico V, Andreola F, Rodriguez de la Concepcion ML, Collins SJ, De Luca LM. Retinoic acid and 4-hydroxyphenylretinamide induce growth inhibition and tissue transglutaminase through different signal transduction pathways in mouse fibroblasts (NIH 3T3 cells). *Carcinogenesis* 1999;20:1133–5.
- [27] Melino G, Thiele CJ, Knight RA, Piacentini M. Retinoids and the control of growth/death decisions in human neuroblastoma cell lines. *J Neurooncol* 1997;31:65–83.
- [28] el-Metwally TH, Hussein MR, Pour PM, Kuszynski CA, Adrian TE. Natural retinoids inhibit proliferation and induce apoptosis in pancreatic cancer cells previously reported to be retinoid resistant. *Cancer Biol Ther* 2005;4:474–83.
- [29] Perry MJ, Mahoney SA, Haynes LW. Transglutaminase C in cerebellar granule neurons: regulation and localization of substrate cross-linking. *Neuroscience* 1995;65:1063–76.
- [30] Campisi A, Bramanti V, Caccamo D, Li Volti G, Cannavo G, Curro M, et al. Effect of growth factors and steroids on transglutaminase activity and expression in primary astroglial cell cultures. *J Neurosci Res* 2008;86:1297–305.
- [31] Fukuda K. Induction of tissue transglutaminase expression by propionate and n-butyrate in colon cancer cell lines. *J Nutr Biochem* 1999;10:397–404.
- [32] Bollag WB, Zhong X, Dodd ME, Hardy DM, Zheng X, Allred WT. Phospholipase D signaling and extracellular signal-regulated kinase-1 and -2 phosphorylation (activation) are required for maximal phorbol ester-induced transglutaminase activity, a marker of keratinocyte differentiation. *J Pharmacol Exp Ther* 2005;312:1223–31.
- [33] Han JA, Park SC. Hydrogen peroxide mediates doxorubicin-induced transglutaminase 2 expression in PC-14 human lung cancer cell line. *Exp Mol Med* 1999;31:83–8.
- [34] Ishii I, Ui M. Possible involvement of GTP-binding proteins in 1 alpha, 25-dihydroxyvitamin D3 induction of tissue transglutaminase in mouse peritoneal macrophages. *Biochem Biophys Res Commun* 1994;203:1773–80.
- [35] Soehnlein O, Eskafi S, Schmeisser A, Kloos H, Daniel WG, Garlisch CD. Atorvastatin induces tissue transglutaminase in human endothelial cells. *Biochem Biophys Res Commun* 2004;322:105–9.
- [36] Shin DM, Jeon JH, Kim CW, Cho SY, Lee HJ, Jang GY, et al. TGFβ mediates activation of transglutaminase 2 in response to oxidative stress that leads to protein aggregation. *Faseb J* 2008.
- [37] Caccamo D, Curro M, Cannavo G, Ientile R. Tissue transglutaminase was up-regulated by EGF-retinoid interplay in epithelial carcinoma cells. *Life Sci* 2004;75:2643–52.
- [38] Ikura K, Shinagawa R, Suto N, Sasaki R. Increase caused by interleukin-6 in promoter activity of guinea pig liver transglutaminase gene. *Biosci Biotechnol Biochem* 1994;58:1540–1.
- [39] Kim SY, Jeong EJ, Steinert PM. IFN-γ induces transglutaminase 2 expression in rat small intestinal cells. *J Interferon Cytokine Res* 2002;22:677–82.
- [40] Giandomenico V, Lancillotti F, Fiorucci G, Percario ZA, Rivabene R, Malorni W, et al. Retinoic acid and IFN inhibition of cell proliferation is associated with apoptosis in squamous carcinoma cell lines: role of IRF-1 and TGase II-dependent pathways. *Cell Growth Differ* 1997;8:91–100.
- [41] Kuncio GS, Tsyganskaya M, Zhu J, Liu SL, Nagy L, Thomazy V, et al. TNF-α modulates expression of the tissue transglutaminase gene in liver cells. *Am J Physiol* 1998;274:G240–5.
- [42] Traher PG. Differentiation of intestinal epithelial cells: lessons from the study of intestine-specific gene expression. *J Lab Clin Med* 1994;123:467–77.
- [43] Massague J, Gomis RR. The logic of TGFβ signaling. *FEBS Lett* 2006;580:2811–20.
- [44] Halttunen T, Maki M. Serum immunoglobulin A from patients with celiac disease inhibits human T84 intestinal crypt epithelial cell differentiation. *Gastroenterology* 1999;116:566–72.
- [45] Barnard JA, Beauchamp RD, Coffey RJ, Moses HL. Regulation of intestinal epithelial cell growth by transforming growth factor type beta. *Proc Natl Acad Sci U S A* 1989;86:1578–82.
- [46] Szondy Z, Sarang Z, Molnar P, Nemeth T, Piacentini M, Mastroberardino PG, et al. Transglutaminase 2<sup>−/−</sup> mice reveal a phagocytosis-associated crosstalk between macrophages and apoptotic cells. *Proc Natl Acad Sci U S A* 2003;100:7812–7.
- [47] Giovannini C, Matarrese P, Scazzocchio B, Vari R, D'Archivio M, Straface E, et al. Wheat gliadin induces apoptosis of intestinal cells via an autocrine mechanism involving Fas-Fas ligand pathway. *FEBS Lett* 2003;540:117–24.
- [48] Elli L, Dolfini E, Bardella MT. Gliadin cytotoxicity and in vitro cell cultures. *Toxicol Lett* 2003;146:1–8.
- [49] Edelblum KL, Yan F, Yamaoka T, Polk DB. Regulation of apoptosis during homeostasis and disease in the intestinal epithelium. *Inflamm Bowel Dis* 2006;12:413–24.
- [50] Bergamini CM, Eckert RL, Ichinose A, Muszbek L, Squerzanti M. Cellular differentiation and death in a renaissance castle. *Cell Death Differ* 2003;10:262–5.
- [51] Piredda L, Amendola A, Colizzi V, Davies PJ, Farrace MG, Fraziano M, et al. Lack of 'tissue' transglutaminase protein cross-linking leads to leakage of macro-
- molecules from dying cells: relationship to development of autoimmunity in MRL<sup>lpr/lpr</sup> mice. *Cell Death Differ* 1997;4:463–72.
- [52] Gentile V, Thomazy V, Piacentini M, Fesus L, Davies PJ. Expression of tissue transglutaminase in Balb-C 3T3 fibroblasts: effects on cellular morphology and adhesion. *J Cell Biol* 1992;119:463–74.
- [53] Milakovic T, Tucholski J, McCoy E, Johnson GV. Intracellular localization and activity state of tissue transglutaminase differentially impacts cell death. *J Biol Chem* 2004;279:8715–22.
- [54] Oliverio S, Amendola A, Rodolfo C, Spinedi A, Piacentini M. Inhibition of "tissue" transglutaminase increases cell survival by preventing apoptosis. *J Biol Chem* 1999;274:34123–8.
- [55] Tucholski J, Lesort M, Johnson GV. Tissue transglutaminase is essential for neurite outgrowth in human neuroblastoma SH-SY5Y cells. *Neuroscience* 2001;102:481–91.
- [56] Buommino E, Pasquali D, Sinisi AA, Bellastella A, Morelli F, Metafora S. Sodium butyrate/retinoic acid costimulation induces apoptosis-independent growth arrest and cell differentiation in normal and ras-transformed seminal vesicle epithelial cells unresponsive to retinoic acid. *J Mol Endocrinol* 2000;24:83–94.
- [57] Antonyak MA, Singh US, Lee DA, Boehm JE, Combs C, Zgola MM, et al. Effects of tissue transglutaminase on retinoic acid-induced cellular differentiation and protection against apoptosis. *J Biol Chem* 2001;276:33582–7.
- [58] Robitaille K, Daviau A, Tucholski J, Johnson GV, Rancourt C, Blouin R. Tissue transglutaminase triggers oligomerization and activation of dual leucine zipper-bearing kinase in calphostin C-treated cells to facilitate apoptosis. *Cell Death Differ* 2004;11:542–9.
- [59] Hebert SS, Daviau A, Grondin G, Latreille M, Aubin RA, Blouin R. The mixed lineage kinase DLK is oligomerized by tissue transglutaminase during apoptosis. *J Biol Chem* 2000;275:32482–90.
- [60] Peng X, Zhang Y, Zhang H, Graner S, Williams JF, Levitt ML, et al. Interaction of tissue transglutaminase with nuclear transport protein importin-α3. *FEBS Lett* 1999;446:35–9.
- [61] Oliverio S, Amendola A, Di Sano F, Farrace MG, Fesus L, Nemes Z, et al. Tissue transglutaminase-dependent posttranslational modification of the retinoblastoma gene product in promonocytic cells undergoing apoptosis. *Mol Cell Biol* 1997;17:6040–8.
- [62] Boehm JE, Singh U, Combs C, Antonyak MA, Cerione RA. Tissue transglutaminase protects against apoptosis by modifying the tumor suppressor protein p110 Rb. *J Biol Chem* 2002;277:20127–30.
- [63] Mishra S, Melino G, Murphy LJ. Transglutaminase 2 kinase activity facilitates protein kinase A-induced phosphorylation of retinoblastoma protein. *J Biol Chem* 2007;282:18108–15.
- [64] Antonyak MA, Boehm JE, Cerione RA. Phosphoinositide 3-kinase activity is required for retinoic acid-induced expression and activation of the tissue transglutaminase. *J Biol Chem* 2002;277:14712–6.
- [65] Cerione RA, Zheng Y. The Dbl family of oncogenes. *Curr Opin Cell Biol* 1996;8:216–22.
- [66] Verma A, Mehta K. Tissue transglutaminase-mediated chemoresistance in cancer cells. *Drug Resist Updat* 2007;10:144–51.
- [67] Nunes I, Gleizes PE, Metz CN, Rifkin DB. Latent transforming growth factor-beta binding protein domains involved in activation and transglutaminase-dependent cross-linking of latent transforming growth factor-beta. *J Cell Biol* 1997;136:1151–63.
- [68] Sohn J, Kim TI, Yoon YH, Kim JY, Kim SY. Novel transglutaminase inhibitors reverse the inflammation of allergic conjunctivitis. *J Clin Invest* 2003;111:121–8.
- [69] Glas J, Torok HP, Tonenchi L, Muller-Myhsok B, Mussack T, Wetzke M, et al. Role of the NFKB1-94ins/delATTG promoter polymorphism in IBD and potential interactions with polymorphisms in the CARD15/NOD2, IKBL, and IL-1RN genes. *Inflamm Bowel Dis* 2006;12:606–11.
- [70] Zaph C, Troy AE, Taylor BC, Berman-Booty LD, Guild KJ, Du Y, et al. Epithelial-cell-intrinsic IKK-beta expression regulates intestinal immune homeostasis. *Nature* 2007;446:552–6.
- [71] Park SS, Kim JM, Kim DS, Kim IH, Kim SY. Transglutaminase 2 mediates polymer formation of I-κBα through C-terminal glutamine cluster. *J Biol Chem* 2006;281:34965–72.
- [72] Falasca L, Farrace MG, Rinaldi A, Tuosto L, Melino G, Piacentini M. Transglutaminase type II is involved in the pathogenesis of endotoxemic shock. *J Immunol* 2008;180:2616–24.
- [73] Maiuri L, Luciani A, Giardino I, Raia V, Vilella VR, D'Apolito M, et al. Tissue transglutaminase activation modulates inflammation in cystic fibrosis via PPARγ down-regulation. *J Immunol* 2008;180:7697–705.
- [74] Mohan K, Pinto D, Issekutz TB. Identification of tissue transglutaminase as a novel molecule involved in human CD8<sup>+</sup> T cell transendothelial migration. *J Immunol* 2003;171:3179–86.
- [75] Schuppan D, Dennis MD, Kelly CP. Celiac disease: epidemiology, pathogenesis, diagnosis, and nutritional management. *Nutr Clin Care* 2005;8:54–69.
- [76] Marsh MN. Gluten, major histocompatibility complex, and the small intestine. A molecular and immunobiological approach to the spectrum of gluten sensitivity ("celiac sprue"). *Gastroenterology* 1992;102:330–54.
- [77] Bardella MT, Trovato C, Cesana BM, Pagliari C, Gebbia C, Peracchi M. Serological markers for coeliac disease: is it time to change? *Dig Liver Dis* 2001;33:426–31.
- [78] Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med* 1997;3:797–801.
- [79] Schuppan D. Current concepts of celiac disease pathogenesis. *Gastroenterology* 2000;119:234–42.



- [80] Shan L, Molberg O, Parrot I, Hausch F, Filiz F, Gray GM, et al. Structural basis for gluten intolerance in celiac sprue. *Science* 2002;297:2275–9.
- [81] Schuppan D, Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, et al. Identification of the autoantigen of celiac disease. *Ann N Y Acad Sci* 1998;859:121–6.
- [82] 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;279:17607–16.
- [83] Kagnoff MF. Celiac disease: pathogenesis of a model immunogenetic disease. *J Clin Invest* 2007;117:41–9.
- [84] Dieterich W, Trapp D, Esslinger B, Leidenberger M, Piper J, Hahn E, et al. Autoantibodies of patients with coeliac disease are insufficient to block tissue transglutaminase activity. *Gut* 2003;52:1562–6.
- [85] Preisz K, Sardy M, Horvath A, Karpatis S. Immunoglobulin, complement and epidermal transglutaminase deposition in the cutaneous vessels in dermatitis herpetiformis. *J Eur Acad Dermatol Venerol* 2005;19:74–9.
- [86] Dieterich W, Esslinger B, Trapp D, Hahn E, Huff T, Seilmeier W, et al. Cross linking to tissue transglutaminase and collagen favours gliadin toxicity in coeliac disease. *Gut* 2006;55:478–84.
- [87] Wang JY, Viar MJ, Johnson LR. Transglutaminase in response to hypertonic NaCl-induced gastric mucosal injury in rats. *Gastroenterology* 1993;104:65–74.
- [88] McCormack SA, Wang JY, Viar MJ, Tague L, Davies PJ, Johnson LR. Polyamines influence transglutaminase activity and cell migration in two cell lines. *Am J Physiol* 1994;267:C706–14.
- [89] Wang JY, Viar MJ, Li J, Shi HJ, Patel AR, Johnson LR. Differences in transglutaminase mRNA after polyamine depletion in two cell lines. *Am J Physiol* 1998;274:C522–30.
- [90] Bowness JM, Tarr AH. Increase in transglutaminase and its extracellular products in response to an inflammatory stimulus by lipopolysaccharide. *Mol Cell Biochem* 1997;169:157–63.
- [91] Jeon JH, Kim IG. Role of protein modifications mediated by transglutaminase 2 in human viral diseases. *Front Biosci* 2006;11:221–31.
- [92] Telci D, Griffin M. Tissue transglutaminase (TG2)—a wound response enzyme. *Front Biosci* 2006;11:867–82.
- [93] Elli L. Coeliac disease: between “pizza” and ethics. *Gut* 2006;55:1672.
- [94] Wong RC, Steele RH, Reeves GE, Wilson RJ, Pink A, Adelstein S. Antibody and genetic testing in coeliac disease. *Pathology* 2003;35:285–304.
- [95] Lindfors K, Kaukinen K, Maki M. A role for anti-transglutaminase 2 autoantibodies in the pathogenesis of coeliac disease? *Amino Acids* 2008.
- [96] Schuppan D, Kelly CP. Is duodenal biopsy required in all patients with suspected celiac disease? *Nat Clin Pract Gastroenterol Hepatol* 2008;5:70–1.
- [97] Burgin-Wolff A, Dahlbom I, Hadziselimovic F, Petersson CJ. Antibodies against human tissue transglutaminase and endomysium in diagnosing and monitoring coeliac disease. *Scand J Gastroenterol* 2002;37:685–91.
- [98] Vahedi K, Mascart F, Mary JY, Laberrenne JE, Bouhnik Y, Morin MC, et al. Reliability of antitransglutaminase antibodies as predictors of gluten-free diet compliance in adult celiac disease. *Am J Gastroenterol* 2003;98:1079–87.
- [99] Reeves GE, Squance ML, Duggan AE, Murugasu RR, Wilson RJ, Wong RC, et al. Diagnostic accuracy of coeliac serological tests: a prospective study. *Eur J Gastroenterol Hepatol* 2006;18:493–501.
- [100] Falini ML, Elli L, Caramanico R, Bardella MT, Terrani C, Roncoroni L, et al. Immunoreactivity of antibodies against transglutaminase-deamidated gliadins in adult celiac disease. *Dig Dis Sci* 2008.
- [101] Rashtak S, Ettore MW, Homburger HA, Murray JA. Comparative usefulness of deamidated gliadin antibodies in the diagnosis of celiac disease. *Clin Gastroenterol Hepatol* 2008;6:426–32, quiz 370.
- [102] Bamias G, Cominelli F. Immunopathogenesis of inflammatory bowel disease: current concepts. *Curr Opin Gastroenterol* 2007;23:365–9.
- [103] Podolsky DK. Inflammatory bowel disease. *N Engl J Med* 2002;347:417–29.
- [104] D’Argenio G, Cicacci C, Sorrentini I, Iovino P, Gatto A, Cosenza V, et al. Serum transglutaminase in inflammatory bowel diseases. *J Clin Gastroenterol* 1990;12:400–4.
- [105] D’Argenio G, Cosenza V, Riegler G, Della Valle N, Deritis F, Mazzacca G. Serum transglutaminase correlates with endoscopic and histopathologic grading in patients with ulcerative colitis. *Dig Dis Sci* 2001;46:649–57.
- [106] Hitomi K. Transglutaminases in skin epidermis. *Eur J Dermatol* 2005;15:313–9.
- [107] D’Argenio G, Grossman A, Cosenza V, Valle ND, Mazzacca G, Bishop PD. Recombinant factor XIII improves established experimental colitis in rats. *Dig Dis Sci* 2000;45:987–97.
- [108] Lorenz R, Born P, Classen M. A prospective pilot study. Substitution of factor XIII concentrate in treatment refractory ulcerative colitis. *Med Klin (Munich)* 1994;89:534–7.
- [109] Lorenz R, Heinmuller M, Classen M, Tornieporth N, Gain T. Substitution of factor XIII: a therapeutic approach to ulcerative colitis. *Haemostasis* 1991;21:5–9.
- [110] Bregenzer N, Lange A, Furst A, Gross V, Scholmerich J, Andus T. Patient education in inflammatory bowel disease does not influence patients knowledge and long-term psychosocial well-being. *Z Gastroenterol* 2005;43:367–71.
- [111] Leeds JS, Horoldt BS, Sidhu R, Hopper AD, Robinson K, Toulson B, et al. Is there an association between coeliac disease and inflammatory bowel diseases? A study of relative prevalence in comparison with population controls. *Scand J Gastroenterol* 2007;42:1214–20.
- [112] Sjober K, Eriksson S, Tenngart B, Roth EB, Leffler H, Stenberg P. Factor XIII and tissue transglutaminase antibodies in coeliac and inflammatory bowel disease. *Autoimmunity* 2002;35:357–64.
- [113] Tursi A. Anti-tissue transglutaminase in inflammatory bowel diseases: an activity disease-related phenomenon? *Dig Liver Dis* 2006;38:711–2.
- [114] Di Tola M, Sabbatella L, Anania MC, Viscido A, Caprilli R, Pica R, et al. Anti-tissue transglutaminase antibodies in inflammatory bowel disease: new evidence. *Clin Chem Lab Med* 2004;42:1092–7.
- [115] Bizzaro N, Villalta D, Tonutti E, Doria A, Tampona M, Bassetti D, et al. IgA and IgG tissue transglutaminase antibody prevalence and clinical significance in connective tissue diseases, inflammatory bowel disease, and primary biliary cirrhosis. *Dig Dis Sci* 2003;48:2360–5.
- [116] Korponay-Szabo IR, Szabados K, Pusztai J, Uhrin K, Ludmany E, Nemes E, et al. Population screening for coeliac disease in primary care by district nurses using a rapid antibody test: diagnostic accuracy and feasibility study. *Br Med J* 2007;335:1244–7.
- [117] Menardo G, Brizzolara R, Bonassi S, Marchetti A, Dante GL, Pistone C, et al. Population screening for coeliac disease in a low prevalence area in Italy. *Scand J Gastroenterol* 2006;41:1414–20.
- [118] Tommasini A, Not T, Kiren V, Baldas V, Santon D, Trevisiol C, et al. Mass screening for coeliac disease using antihuman transglutaminase antibody assay. *Arch Dis Child* 2004;89:512–5.
- [119] Catassi C, Ratsch IM, Fabiani E, Ricci S, Bordinchia F, Pierdomenico R, et al. High prevalence of undiagnosed coeliac disease in 5280 Italian students screened by anti-gliadin antibodies. *Acta Paediatr* 1995;84:672–6.
- [120] Cuzzocrea S, McDonald MC, Mazzon E, Mota-Filipe H, Centorino T, Terranova ML, et al. Calpain inhibitor I reduces colon injury caused by dinitrobenzene sulphonic acid in the rat. *Gut* 2001;48:478–88.
- [121] D’Argenio G, Farrace MG, Cosenza V, De Ritis F, Della Valle N, Manguso F, et al. Expression of apoptosis-related proteins in rat with induced colitis. *Int J Colorectal Dis* 2004;19:451–60.
- [122] Schuppan D, Afdhal NH. Liver cirrhosis. *Lancet* 2008;371:838–51.
- [123] Wallace K, Burt AD, Wright MC. Liver fibrosis. *Biochem J* 2008;411:1–18.
- [124] Schuppan D, Ruehl M, Somasundaram R, Hahn EG. Matrix as a modulator of hepatic fibrogenesis. *Semin Liver Dis* 2001;21:351–72.
- [125] Facchiano F, Facchiano A, Facchiano AM. The role of transglutaminase-2 and its substrates in human diseases. *Front Biosci* 2006;11:1758–73.
- [126] Facchiano F, Facchiano A. Transglutaminases and their substrates. *Prog Exp Tumor Res* 2005;38:37–57.
- [127] Grenard P, Bresson-Hadni S, El Alaoui S, Chevallier M, Vuitton DA, Ricard-Blum S. Transglutaminase-mediated cross-linking is involved in the stabilization of extracellular matrix in human liver fibrosis. *J Hepatol* 2001;35:367–75.
- [128] Nardacci R, Ciccosanti F, Falasca L, Lo Iacono O, Amendola A, Antonucci G, et al. Tissue transglutaminase in HCV infection. *Cell Death Differ* 2003;10(Suppl. 1):S79–80.
- [129] Issa R, Zhou X, Constandinou CM, Fallowfield J, Millward-Sadler H, Gaca MD, et al. Spontaneous recovery from micronodular cirrhosis: evidence for incomplete resolution associated with matrix cross-linking. *Gastroenterology* 2004;126:1795–808.
- [130] Zhou X, Jamil A, Nash A, Chan J, Trim N, Iredale JP, et al. Impaired proteolysis of collagen I inhibits proliferation of hepatic stellate cells: implications for regulation of liver fibrosis. *J Biol Chem* 2006;281:39757–65.
- [131] Sottrup-Jensen L, Hansen HF, Pedersen HS, Kristensen L. Localization of epsilon-lysyl-gamma-glutamyl cross-links in five human alpha 2-macroglobulin-proteinase complexes. Nature of the high molecular weight cross-linked products. *J Biol Chem* 1990;265:17727–37.
- [132] Esposito C, Caputo I, Troncone R. New therapeutic strategies for coeliac disease: tissue transglutaminase as a target. *Curr Med Chem* 2007;14:2572–80.
- [133] Li JY, Popovic N, Brundin P. The use of the R6 transgenic mouse models of Huntington’s disease in attempts to develop novel therapeutic strategies. *NeuroRx* 2005;2:447–64.
- [134] Sollid LM, Khosla C. Future therapeutic options for celiac disease. *Nat Clin Pract Gastroenterol Hepatol* 2005;2:140–7.
- [135] Kim SY. New target against inflammatory diseases: transglutaminase 2. *Arch Immunol Ther Exp (Warsz)* 2004;52:332–7.
- [136] Wilhelmus MM, van Dam AM, Drukarch B. Tissue transglutaminase: a novel pharmacological target in preventing toxic protein aggregation in neurodegenerative diseases. *Eur J Pharmacol* 2008;585:464–72.
- [137] Lorand L, Conrad SM. Transglutaminases. *Mol Cell Biochem* 1984;58:9–35.
- [138] Lai TS, Slaughter TF, Peoples KA, Hettasch JM, Greenberg CS. Regulation of human tissue transglutaminase function by magnesium-nucleotide complexes. Identification of distinct binding sites for Mg-GTP and Mg-ATP. *J Biol Chem* 1998;273:1776–81.
- [139] Aeschlimann D, Paulsson M. Transglutaminases: protein cross-linking enzymes in tissues and body fluids. *Thromb Haemost* 1994;71:402–15.
- [140] Duval E, Case A, Stein RL, Cuny GD. Structure-activity relationship study of novel tissue transglutaminase inhibitors. *Bioorg Med Chem Lett* 2005;15:1885–9.
- [141] Case A, Stein RL. Kinetic analysis of the interaction of tissue transglutaminase with a nonpeptidic slow-binding inhibitor. *Biochemistry* 2007;46:1106–15.
- [142] de Macedo P, Marrano C, Keillor JW. A direct continuous spectrophotometric assay for transglutaminase activity. *Anal Biochem* 2000;285:16–20.
- [143] Killackey JJ, Bonaventura BJ, Castelano AL, Billedeau RJ, Farmer W, DeYoung L, et al. A new class of mechanism-based inhibitors of transglutaminase enzymes inhibits the formation of cross-linked envelopes by human malignant keratinocytes. *Mol Pharmacol* 1989;35:701–6.

- [144] Marrano C, de Macedo P, Gagnon P, Lapierre D, Gravel C, Keillor JW. Synthesis and evaluation of novel dipeptide-bound 1,2,4-thiadiazoles as irreversible inhibitors of guinea pig liver transglutaminase. *Bioorg Med Chem* 2001;9:3231–41.
- [145] de Macedo P, Marrano C, Keillor JW. Synthesis of dipeptide-bound epoxides and alpha,beta-unsaturated amides as potential irreversible transglutaminase inhibitors. *Bioorg Med Chem* 2002;10:355–60.
- [146] Hausch F, Halttunen T, Maki M, Khosla C. Design, synthesis, and evaluation of gluten peptide analogs as selective inhibitors of human tissue transglutaminase. *Chem Biol* 2003;10:225–31.
- [147] Jeitner TM, Delikatny EJ, Ahlqvist J, Capper H, Cooper AJ. Mechanism for the inhibition of transglutaminase 2 by cystamine. *Biochem Pharmacol* 2005;69:961–70.
- [148] Molberg O, McAdam S, Lundin KE, Kristiansen C, Arentz-Hansen H, Kett K, et al. T cells from celiac disease lesions recognize gliadin epitopes deamidated in situ by endogenous tissue transglutaminase. *Eur J Immunol* 2001;31:1317–23.
- [149] Maiuri L, Ciacchi C, Ricciardelli I, Vacca L, Raia V, Rispo A, et al. Unexpected role of surface transglutaminase type II in celiac disease. *Gastroenterology* 2005;129:1400–13.
- [150] Strnad P, Siegel M, Toivola DM, Choi K, Kosek JC, Khosla C, et al. Pharmacologic transglutaminase inhibition attenuates drug-primed liver hypertrophy but not Mallory body formation. *FEBS Lett* 2006;580:2351–7.
- [151] Choi K, Siegel M, Piper JL, Yuan L, Cho E, Strnad P, et al. Chemistry and biology of dihydroisoxazole derivatives: selective inhibitors of human transglutaminase 2. *Chem Biol* 2005;12:469–75.
- [152] Qiu JF, Zhang ZQ, Chen W, Wu ZY. Cystamine ameliorates liver fibrosis induced by carbon tetrachloride via inhibition of tissue transglutaminase. *World J Gastroenterol* 2007;13:4328–32.
- [153] Lesort M, Lee M, Tucholski J, Johnson GV. Cystamine inhibits caspase activity. Implications for the treatment of polyglutamine disorders. *J Biol Chem* 2003;278:3825–30.
- [154] Jokay I, Kelemenics K, Gyuris A, Minarovits J. S-methylthio-cysteine and cystamine are potent stimulators of thiol production and glutathione synthesis. *Life Sci* 1998;62:PL27–33.
- [155] Bailey CD, Johnson GV. The protective effects of cystamine in the R6/2 Huntington's disease mouse involve mechanisms other than the inhibition of tissue transglutaminase. *Neurobiol Aging* 2006;27:871–9.
- [156] Garattini E, Gianni M, Terao M. Retinoids as differentiating agents in oncology: a network of interactions with intracellular pathways as the basis for rational therapeutic combinations. *Curr Pharm Des* 2007;13:1375–400.
- [157] Mora JR. Homing imprinting and immunomodulation in the gut: role of dendritic cells and retinoids. *Inflamm Bowel Dis* 2008;14:275–89.
- [158] Mucida D, Park Y, Kim G, Turovskaya O, Scott I, Kronenberg M, et al. Reciprocal TH17 and regulatory T cell differentiation mediated by retinoic acid. *Science* 2007;317:256–60.