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REVIEW

Transglutaminase 2 has opposing roles in the regulation of cellular functions as well as cell growth and death

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Transglutaminase 2 (TG2) is primarily known as the most ubiquitously expressed member of the transglutaminase family with Ca^{2+}-dependent protein crosslinking activity; however, this enzyme exhibits multiple additional functions through GTPase, cell adhesion, protein disulfide isomerase, kinase, and scaffold activities and is associated with cell growth, differentiation, and apoptosis. TG2 is found in the extracellular matrix, plasma membrane, cytosol, mitochondria, recycling endosomes, and nucleus, and its subcellular localization is an important determinant of its function. Depending upon the cell type and stimuli, TG2 changes its subcellular localization and biological activities, playing both anti- and pro-apoptotic roles. Increasing evidence indicates that the GTP-bound form of the enzyme (in its closed form) protects cells from apoptosis but that the transamidation activity of TG2 (in its open form) participates in both facilitating and inhibiting apoptosis. A difficulty in the study and understanding of this enigmatic protein is that opposing effects have been reported regarding its roles in the same physiological and/or pathological systems. These include neuroprotective or neurodegenerative effects, hepatic cell growth-promoting or hepatic cell death-inducing effects, exacerbating or having no effect on liver fibrosis, and anti- and pro-apoptotic effects on cancer cells. The reasons for these discrepancies have been ascribed to TG2's multifunctional activities, genetic variants, conformational changes induced by the immediate environment, and differences in the genetic background of the mice used in each of the experiments. In this article, we first report that TG2 has opposing roles like the protagonist in the novel Dr. Jekyll and Mr. Hyde, followed by a summary of the controversies reported, and finally discuss the possible reasons for these discrepancies.
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Facts

- Tissue transglutaminase (TG2) is a multifunctional enzyme that exhibits crosslinking, GTPase, cell adhesion, protein disulfide isomerase, kinase, and scaffold activities.
- By virtue of these multiple activities, TG2 is implicated in the regulation of cell growth, differentiation, and apoptosis.
- However, opposing effects have been reported regarding its roles even in the same physiological and pathological systems.
- To help understand these discrepancies, we summarize and discuss possible reasons for opposing effects in each case.

Open Questions

- How can we measure the individual activity of multiple functions of TG2 *in vivo*?
- How are these multiple functions and genetic variants of TG2 regulated?
- How is TG2's substrate specificity (other than its subloca-lization) determined?

Transglutaminase 2 (TG2) is a multifunctional enzyme and the most ubiquitously expressed member of the large TG family, a protein family of eight isozymes designated as blood coagulation factor XIII and TG1-7. TGs primarily deamidate γ-carboxamide groups of specific protein-bound glutamines while exchanging any primary amines, the ε-amino group of a lysine residue, or water, and form ammonia plus an N^{ε}(γ-glutamyl)lysine crosslinking between glutamine and lysine residues or convert glutamine to glutamic acid.[1–3] TG2 exerts additional enzymatic activities that do not require Ca^{2+};[1,3] that is, hydrolyzing ATP and GTP to mediate signal transduction through G-protein-coupled receptors,[4,5] protein disulfide isomerase and protein kinase,[6,7] interacting with several proteins as an adhesion or scaffold protein[8] (Figure 1).

TG2 is predominantly found in the cytosol. TG2 also appears in the nucleus, mitochondria, plasma membrane, and extracellular matrix (ECM).[9] In mammals, TG2 is widely

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Abbreviations: TG, Transglutaminase; ECM, Extracellular matrix; ER, Endoplasmic reticulum; FN, Fibronectin; NF-κB, Nuclear factor-kappa B; RA, Retinoic acid; RAR, Retinoic acid receptor; PDI, Protein disulfide isomerase; TGF-β, Transforming growth factor-β; ASH, Alcoholic steatohepatitis; NASH, Non-alcoholic steatohepatitis
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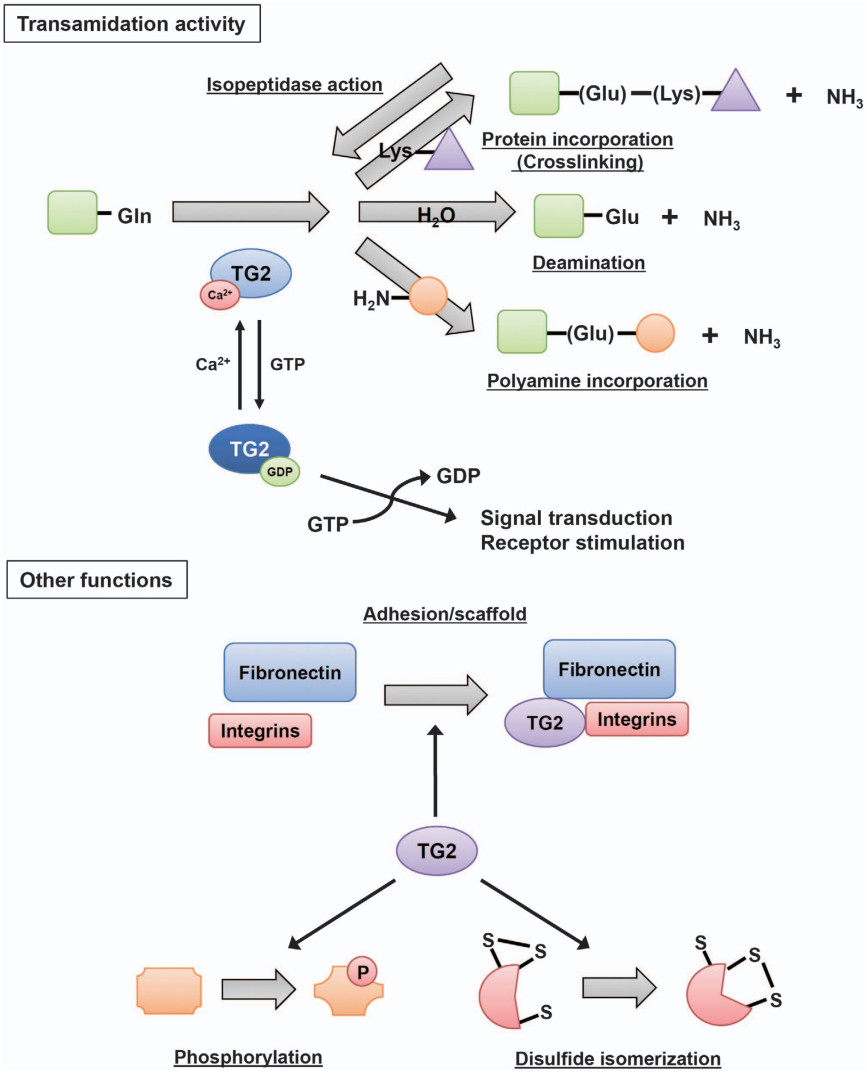

Figure 1 Diverse functions of TG2. TG2 regulates the post-translational modification of several proteins though several activities, including transamidation, GTPase, adhesion/scaffold, kinase, disulfide isomerase, and isopeptidase activities. Some functions of TG2 remain unclear. TG2 exerts different activities depending on the stimuli; these activities lead to several effects, including apoptosis, cell growth, and differentiation

present throughout the body including blood, extracellular spaces, and intracellular compartments of nearly all tissues, and induces tissue remodeling/wound healing and ECM assembly as well as cell growth, differentiation, and cell death.[10] TG2 is thus involved in the pathogenesis/treatment of cancer, diabetes, neurodegeneration, fibrosis, inflammatory, and autoimmune disorders[2] as well as liver diseases.[11–13] For the general functions and structure of TG2, including topics on genetically engineered mouse models and inherited disorders, refer to well-written review articles and/or a book recently released.[2,9,14]

Opposing effects have been reported for the roles of TG2 in the same physiological and/or pathological systems due to differences in

(1) The enzymatic and non-enzymatic activities or properties of TG2, that is, GTPase *versus* transamidation *versus* scaffold activities.[2,15,16]
(2) Genetic variants including alternative splicing, that is, the short or truncated form (TG2-S) *versus* the long form (TG2-L)[17] and single-nucleotide polymorphisms.[18]
(3) The conformational structure (open *versus* closed forms), which is affected by immediate cellular and tissue environments.[2,15]
(4) The genetic background of mice.[2,13]

The aim of this review is to summarize recently obtained knowledge of how TG2 plays Dr. Jekyll and Mr. Hyde (opposing roles) in the regulation of cell growth and death.

Multifunctional Activities of TG2 and Its Regulation

GTPase. Intracellular TG2 in its closed form acts as a Ca^{2+}-independent GTPase in normal cells when the intracellular Ca^{2+} concentration is as low as 10–20 nM, participating

Table 1 List of factors reported to regulate TG2 expression and activity

Name	Cell types	Phenotypes
Ethanol	Hepatocytes	Hepatic injury[12]
Retinoid (retinoic acid) and retinoid receptors	Most cell types, including hepatocytes, hepatocellular carcinoma cells, endothelial cells, leukemia cells, and neuronal cells	Apoptosis,[54,75] differentiation,[56,173] chemoresistance[47]
Free fatty acids	Hepatocytes	Hepatic injury, lipid accumulation[11]
IL-1	Meniscal cells	Inflammation[51]
IL-6	Hepatoblastoma cells	Inflammation[52]
NF-κB	Hepatocytes, breast cancer cells, neuronal cells	Hepatic injury,[11,118] hepatic fibrogenesis,[118] chemoresistance[41,49]
TNF-α	Hepatocytes	Hepatic fibrogenesis[53]
TGF-β/bone morphogenic protein 4	Epithelial-like cells from mink lung, preosteoblastic cells	Not examined
Nitric oxide	Endothelial cells, neuronal cells	Vascular stiffness,[68] neurodegenerative disease[81]
Protein inhibitor of activated STAT (PIASy), E3 SUMO-protein ligase	Bronchial epithelial cells with cystic fibrosis, lung carcinoma	Autophagy[174]
miR-19	Colorectal cancer	Downregulation of TG2 leads to enhanced invasion and metastasis[18]

in the transmembrane signaling of phospholipase Cδ as a component of α_1-adrenergic receptor complexes and supporting the growth of hepatic cells.[16,19,20] GTP binding with TG2 sustains the closed form and prevents its conformational change to the open form; the emergence of crosslinking activity is thus a result of binding to Ca^{2+}, whereas the depletion of GTP causes conformational changes in the open form. In this scenario, the emergence of crosslinking activity induces apoptosis in islet β-cells[21] and other cell types.[22]

Transamidase. When cells are injured (or receive certain stimuli) and the intracellular Ca^{2+} concentration increases to more than 700–800 nM, TG2 markedly alters its structure and transforms into an open form that exerts crosslinking activity.[16,23] In the specific conditions such as tissue regeneration and remodeling, TG2 crosskinks ECM proteins, however, despite a high extracellular Ca^{2+} concentration (in the mM range) outside of cells, TG2 usually does not exert transamidation but instead mediates multiple and complex regulatory interactions, particularly in the regulation of cell-ECM interactions and outside-in signaling via transmembrane receptors, indicating that Ca^{2+} and/or GTP concentrations are not the sole determinants of transamidation activity.

Integrin–fibronectin interaction. TG2 has been identified as an important extracellular crosslinking enzyme involved in ECM turnover.[24–27] In contrast, TG2 is secreted into ECM and forms a hetero complex with its high-affinity binding partner fibronectin (FN) through its N-terminal 42-kD fragment in a crosslinking activity-independent manner.[28,29] The TG–FN complex promotes FN fibril deposition and RGD-independent cell adhesion via syndecan-4/2 and $\alpha5\beta1$ integrin co-signaling,[29] and sustains cell survival in osteoblasts,[30] bone marrow-derived mesenchymal stem cells,[31] and many tumor cells.[32,33]

Miscellaneous functions. Protein disulfide isomerase (PDI) activity[6] has been implicated in mitochondrial-dependent apoptosis,[34] whereas TG2 has been reported to exert an intrinsic serine/threonine kinase activity and to phosphorylate insulin-like growth factor (IGF)-binding protein-3 (IGFBP3),[7] p53 tumor suppressor protein,[35] H1-4 histones,[36] and Rb protein.[37] Moreover, TG2 affects the hypusination of eukaryotic initiation factor 5A (eIF5a) in BALB/c 3T3 cells;[38] this action may be related to the fact that eIF5a serves as a binding protein and/or substrate for TG2.[39,40]

Regulation. The gene expression related to TG2 is modulated by endoplasmic reticulum (ER) stress,[11,41] tissue remodeling,[42] inflammation,[2,43] viral infection,[44] and apoptotic signals,[45,46] as well as cancers[2,47–49] and mediated by soluble factors such as transforming growth factor (TGF)-β/bone morphogenetic protein 4,[50] interleukin (IL)-1,[51] IL-6,[52] tumor necrosis factor (TNF)-α,[53] and epidermal growth factor (EGF)[47] (Table 1). Retinoic acid (RA) induces TG2 expression;[54,55] in acute promyelocytic leukemia treated with RA, TG2 has an important role in the neutrophil–granulocyte differentiation and gene expression of neutrophil's cellular functions in addition to related adhesive, migratory, and phagocytic capacities.[56] The transcriptional activation of the TG2 gene, *TGM2*, is mediated by nuclear factor-kappa B (NF-κB),[41] RA receptor (RAR)/retinoid X receptor, liver X receptor/RAR, and Sp1.[55]

Two examples of a positive feedback loop are known to act in the regulation of TG2 expression. TG2 is indispensable for latent TGF-β activation in many tissues,[57–63] and the generation of TGF-β may stimulate TG2 gene expression. TG2 activates NF-κB via the depletion of inhibitor of κB (I-κB)α via polymerization in the absence of I-κBα kinase activation. This activity results in the dissociation of NF-κB and its translocation to the nucleus, where it may upregulate TG2 in cancer cells.[64–66]

Ca^{2+} and nucleotides (GTP/ATP), respectively, act as the activator and suppressors of the transamidase activity of TG2. Nitric oxide (NO) is also a potent inhibitor by promoting S-nitrosylation on the active site C277.[67] The decreased S-nitrosylation of TG2 contributes to age-related increases in vascular stiffness.[68] Micro-RNA (miR-19) directly downregulates TG2 expression and enhances the invasion of colorectal cancer cells.[18] Finally, the SUMOylation of TG2 enhances its

Table 2 Examples of opposing functions of TG2 in the brain

Brain	Reports	Possible causation	References
Protective effect	Hypoxia-induced cell death in ischemic stroke Infarct volume in human TG2-expressing mice was reduced.	Cell proliferation of GTPase activity is enhanced by neuronal TG2 overexpression and nuclear localization.	84
Degenerative effect	Huntington's disease in R6/1 transgenic mice Polyglutamine disease in spinocerebellar ataxia-1 transgenic mice Crosslinking of huntingtin with expanded polyglutamine, β-amyloid, tau, and α-synuclein in Huntington's, Alzheimer's, and Parkinson's diseases Cell death in ischemic stroke Infarct volume in TG2$^{-/-}$ mice was reduced.	Genetic knockout or inhibitor of TG2 attenuates neurodegenerative disease regardless of whether TG2 is involved in the formation of polyglutamine. The open conformation of TG2 is toxic in cytosol, whereas the closed conformation of TG2 promotes survival in the nucleus.	96,97,175,176 177 88,114,175,178,179 82,180
	Short form of TG2	The short form of TG2 induces cell damage and death	91

protein levels and activity by blocking the ubiquitination of (and thus stabilizing) TG2 protein.[69]

Opposing Roles of TG2 *In Vitro*

The accumulation of TG2 in various cell and tissue types undergoing apoptosis has been demonstrated;[70,71] elevated TG2 activity is correlated with enhanced apoptosis in a neuroblastoma cell line,[72] whereas treatment with antisense against TG2 reduces apoptosis.[73] When neuroblastoma cells are transfected with plasmid overexpressing TG2 but not mutated TG2 lacking crosslinking activity and then treated with staurosporine or osmotic stress, an increased crosslinking activity accompanies the activation of caspase-3 and apoptotic nuclear changes.[74] In mitochondria, interaction with Bax through TG2's BH3 domain plus the predicted crosslinking of Bax causes mitochondrial depolarization, the release of cytochrome *c*, and cell death.[45] Nuclear TG2 causes caspase-independent cell death via the crosslinking and inactivation of the general transcription factor Sp1, resulting in the reduced expression of growth factor receptors, such as c-Met and EGF receptors, that are essential for cell survival.[12,75] In contrast, numerous reports have found a protective effect of TG2 in cell death induced by TNF-α,[76] RA,[77] and stressors such as thapsigargin, hyperosmotic stress, and oxygen/glucose deprivation.[78]

Below are examples in which TG2 has Dr. Jekyll and Mr. Hyde (opposing functions; Tables 2,3,4,5,6).

Neuroprotective *Versus* Neurodegenerative Roles by Modulating HIF1 and Huntingtin Functions, Respectively

Increased activity and expression of TG2 are observed in the ischemic hippocampus after reperfusion (Table 2).[79,80] TG2 expression is induced by oxidative stress, and the induction of inducible NO synthase and NO production, which contribute to neurodegeneration, are closely associated with TG2 expression in the lipopolysaccharide-stimulated activation of astrocytes.[81] The infarction volume was smaller in both TG2 knockout mice and mice treated with cystamine than in control mice.[82] In contrast, both SH-SY5Y human neuroblastoma cells[83] and a permanent middle cerebral artery ligation stroke model[84] demonstrated that hypoxic conditions increased nuclear TG2, TG2 binding to HIF1β independently of transamidase activity, and the prevention of the upregulation of pro-apoptotic Bnip3[85] and Noxa,[86] thereby protecting neuronal cells from hypoxia-induced death in ischemia and stroke.[83,84]

Increased TG2 levels and/or activity, especially the nvolvement of nuclear TG2, have been observed in many neurodegenerative diseases such as Alzheimer's disease (AD), Huntington's disease (HD), and Parkinson's disease (PD).[87] AD is characterized by the formation of extracellular neurotoxic aggregates consisting of amyloid-β protein or intracellular neurotoxic aggregates consisting of hyperphosphorylated tau. TG2 mediates the crosslinking of both amyloid-β and tau *in vitro*[88,89] and the polyamination of the tau protein, resulting in an increased resistance of tau to proteolytic degradation by calpain. This change leads to higher levels of non-degradable tau within the neuron,[90] which indicates that TG2 may accelerate the aggregation process of amyloid-β and tau in AD patients. Furthermore, the enhanced expression of a short form of TG2 (sTG2), an alternatively spliced transcript lacking GTPase activity that maintains poor crosslinking activity and thus has a pro-apoptotic property, has been reported in AD patients,[22,91,92] whereas TG2 is not a biochemical marker for AD disease because no colocalization of TG2 with tau or amyloid-β deposits is found in neocortex sections.[93]

In the frontal cortex of postmortem HD brain tissues, 99% colocalization is observed between ε-(γ-glutamyl)lysine crosslinks and huntingtin aggregates in the nucleus,[94] indicating an involvement of nuclear TG2 in HD. Furthermore, the TG inhibitors cystamine and monodansylcadaverine partially suppress aggregate formation and apoptosis in cells expressing truncated dentatorubral-pallidoluysian atrophy protein with an expanded polyglutamine stretch.[95] Although, (i) the *in vivo* ablation of the TG2 gene ameliorates HD symptoms and leads to unaltered or even increased numbers of neuronal intranuclear inclusions,[96,97] which has recently been ascribed at least in part to a loss of TG2's regulatory effect on autophagy;[98] (ii) cystamine inhibits not only TG2 but also caspase-3;[99] (iii) neuronal intranuclear inclusions are likely to be formed at a late stage of aggregation and do not directly affect the progression of pathogenesis;[100] and (iv) the genetic deletion or enzymatic inhibition of TG2 alleviates the degenerative process and improves survival as well as life span,[97,101]

Table 3 Examples of opposing functions of TG2 in the liver

Liver	Reports	Possible causation	References
Protective effect	Activation of Sp1 via its dimerization	Difference in cell types	123
	CCl_4 and Fas-induced liver injury		20,128
Promotive effect	CCl_4-induced liver injury and fibrosis	Different animal backgrounds	121
	Liver damage and injury in ASH/alcohol, NASH/FFAs		11,12,136,137
	Liver damage in NZB/W F1 mice (a well-known lupus-prone strain)		139
No effect	CCl_4- or thioacetamide-induced liver fibrosis		141

Table 4 Examples of opposing functions of TG2 in cancer

Cancer	Reports	Possible causation	References
Progressive effect	Increased drug resistance and metastasis in breast cancer cells	Different cell types	33,142
	Stabilization of adhesive interactions for metastasis in melanoma cells	Different cell types	144
	Anti-apoptotic effect in RA- or HPR-treated NIH3T3, HL-60, and embryonic lung fibroblast cells	Retention of Rb protein action	150
	Attenuated apoptosis in thapsigargin-treated HEK293 cells	Different chemopreventive agents and cell types	151
	TG2 enhances EGFR expression and transformation in glioblastomas	Interference with EGFR downregulation	148
Suppressive effect	Suppression of tumor progression and metastasis	Reduced metastatic activity via excess stabilization/accumulation of ECM crosslinking	145,146
	Chemoprevention by retinoid	Different chemopreventive agents and cell types	75
	Growth arrest/apoptosis in RA-treated U937 cells	Lost activity of Rb protein	149
	Phosphorylation of Rb protein in MCF-7		37
No effect	Cell growth/apoptosis in TG2-transfected fibrosarcoma cell	Different cell types	146

Table 5 Examples of opposing functions of TG2 in the pancreas

Pancreas	Reports	Possible causation	References
Promotive effect	Glucose-stimulated insulin release	Different functional activity of TG2	152–154,158,159
	Insulin secretion induced by RA and Ca^{2+}		155,156
No effect	Insulin secretion induced by either cAMP or the phorbol ester PMAIntraperitoneal glucose tolerance tests and insulin tolerance tests	Different functional activity of TG2Different background of TG2 knockout miceBroad biological effects of TG inhibitors	157,160

isolated polyglutamine forms aggregates through a β-sheet-based nucleation mechanism, indicating a causative role for nuclear TG2 in HD pathogenesis via mechanisms other than the formation of neuronal intranuclear inclusions.[102]

Transcription dysregulation and impaired energy homeostasis have important roles. The introduction of ZDON, a peptide inhibitor of TG2, ameliorates HD symptoms.[101,103] The mutant huntingtin binds to and inactivates other polyglutamine-enriched proteins such as transcription factors, including the general transcription factor Sp1 or its coactivator TAFII130,[104–106] which may repress the Sp1-dependent transcription of BDNF,[107] dopamine D2 receptor,[104,105] preproenkephalin,[104] the mitochondrial proteins PGC-1α, and cytochrome c.[108,109] These results imply that although nuclear TG2 is not essential for inducing HD, it may be an important factor in exaggerating HD symptoms through the transcriptional dysregulation of these survival factors and key metabolic genes. Defective nuclear actin remodeling causes faster cell death in correlation with disease progression.[110] Cofilin is an actin binding protein that is required for actin treadmilling.[111] The formation of the dynamic cytoskeleton, referred to as 'actin-cofilin rods', is a self-protecting reaction against cellular stress and is sustained by an association with normal huntingtin upon its release from the ER and localization to the nucleus under stress conditions. In response to cellular stress, delayed and aberrant actin-cofilin rods are formed in neurodegenerative disease by (i) mutant huntingtin via the impairment of the normal function of huntingtin and (ii) an excessive crosslinking of actin-cofilin complexes by stress-activated nuclear TG2.[110] As stated above, TG2 binds

Table 6 Examples of opposing functions of TG2 in blood vessel formation

Blood vessel	Reports	Possible causation	References
Positive role	Formation and stability of blood vessels	Promotion of angiogenic process via VEGF signals in endothelium	161,162
	Migration and tubule formation of HUVEC	TG2 inhibition results in reduced FN deposition, matrix-bound VEGFA, phosphorylation of VEGF receptor 2, and then suppresses migration and tubule formation	30
	Endothelial sprouting, migration of both endothelial and vascular mesenchymal cells, and organization of actin cytoskeleton	Promotion of the angiogenic process in endothelial and mesenchymal cells	164
	Cell spreading and adhesion in the human endothelial cell line ECV304	Adhesion activity with integrin or FN	113,166
	Colocalization and binding with endostatin	Promotion of angiogenesis via endostatin-TG2 binding	167,168
Negative role	Inhibition of angiogenesis and tumor growth via matrix changes through an intratumoral injection of TG2 into mice	Reduced organized vasculature via excess stabilization/accumulation of ECM crosslinking	171
	RA-mediated apoptosis in vascular smooth muscle cells	Effect in smooth muscle cells	54

FN through its N-terminal domain and interacts with many other scaffold proteins such as integrins and lamins A and C.[28,112,113] In PD patients' brains, TG2 expression is increased in the substantia nigra, and TG2-catalyzed crosslinking has been shown to colocalize with α-synuclein, a substrate for TG2 *in vivo*,[114] in dementia with Lewy bodies.[115]

Hepatic Protective *Versus* Insult Roles Through Sp1 Crosslinking

TG2 crosslinking activity significantly increases with carbon tetrachloride (CCl_4) or ethanol-induced liver injury in rats and in acute human liver injury (Table 3).[116–120] Cystamine and garlic extract prevent CCl_4-induced liver injury and fibrosis via the inhibition of TG2.[121] Sp1 is a general transcription factor that is rich in lysine and glutamine residues,[122] thereby serving as a good substrate for TG2 both *in vitro* and *in vivo*.[12] An upregulation of Sp1's transcriptional activation activity upon crosslinking with nuclear TG2 in human 293T cells likely occurred because Sp1 exerts higher transcriptional activation activity as a dimer or trimer,[123,124] whereas in alcohol or free fatty acid (FFA)-treated hepatic cells, highly crosslinked, oligomerized Sp1 loses its transcriptional activation activity. A defect in Sp1 activity causes the decreased expression of *c-Met*, the major receptor for hepatocyte growth factor, leading to caspase-independent hepatic cell death in culture systems and animal models as well as in patients with both alcoholic steatohepatitis (ASH) and non-ASH (NASH).[11,12,75] FFAs increase ER stress, NF-κB activation, and nuclear TG2 through a pancreatic ER kinase (PERK)-dependent pathway, whereas ethanol-induced nuclear TG2 is dependent at least in part on retinoid signaling.[11] RA enhances the transcription of the *TG2* gene via GC box motifs in its promoter region through a physical interaction between newly synthesized RARs and preexisting Sp1.[55] Therefore, the crosslinking and silencing of Sp1 by TG2 may be a feedback mechanism to control the excessive expression of TG2 and prevent it from playing Mr. Hyde.

Furthermore, TG2 contributes to the clearance of apoptotic cells by promoting monocyte infiltration via the dimerization of the monocyte chemotactic factor S19[125] followed by macrophage engulfment via integrin β3,[126] leading to inflammation suppression. In addition, TG2 contributes to wound repair and tissue stabilization via the crosslinking of various intracellular and extracellular proteins at inflammation sites.[42] A peptide with anti-TG2 activity decreases lung inflammation accompanied by reduced neutrophil infiltration and cytokines expression.[127]

However, TG2 has been reported to be anti-apoptotic, and the effect is linked to both its GTP binding and crosslinking activity.[15,20] TG2-null mice do not exhibit an obvious hepatic phenotype defect but do exhibit an impaired clearance of apoptotic cells by phagocytosis under stress conditions and experience a more severe liver injury after CCl_4 or Fas administration.[20,128] This discrepancy can be explained as follows. Sarang *et al.*[20] reported that TG2 protects against high-dose (1 μg/g body weight) Jo2 (anti-Fas antibody)-induced liver injury; $TG2^{-/-}$ mice were more sensitive to Jo2-mediated necrosis than $TG2^{+/+}$ animals. In those studies, Sarang *et al.*[20] used FVB mice as wild-type controls. We used wild-type littermates from heterozygous $TG2^{+/-}$ crosses and applied a high dose of Jo2 (used by Sarang *et al.*[20]); this treatment resulted in massive hepatic necrosis both in $TG2^{+/+}$ and $TG2^{-/-}$ mice. Thus, the varying genetic backgrounds of the $TG2^{+/+}$ *versus* $TG2^{-/-}$ mice used by Sarang *et al.*[20] might have contributed to the discrepancy between their findings and ours. However, we reproduced the protective effect of TG2 silencing on hepatic injury with a low dose (0.1 μg/g body weight) of Jo2 using the exact same $TG2^{-/-}$ mouse line used by Sarang *et al.*[20] Thus, TG2 may promote the hepatic apoptosis caused by relatively low doses (but not higher doses) of Jo2.

Using the same $TG2^{-/-}$ mouse line used by Sarang *et al.*,[20] Nardacci *et al.*[128] reported that TG2 is protective in CCl_4-mediated liver injury and speculated that the observed increase in TG2 expression during the initial stages of liver fibrosis in HCV-infected patients may protect against liver injury. However, another explanation may be that high levels of TG2 contribute to liver injury in these patients; as we have shown in an animal model,[12] TG2 inhibitors may be a useful treatment for the prevention of hepatic apoptosis.

Fibrogenic *Versus* No or anti-Fibrogenic Functions of TG2

In injured liver cells, TG2 transforms into a crosslinking enzyme (open form) and contributes to the wound healing process and fibrosis (Table 3). First, the crosslinking reaction results in the formation of an N^{ε}(γ-glutamyl)lysine isopeptide bond, which is one important step in the maturation or stabilization of ECMs (such as collagens) in the extracellular space, exacerbating hepatic fibrosis.[117,129] The N^{ε}(γ-glutamyl)lysine crosslink, which is undetectable in normal liver tissue, is present extracellularly in the fibrotic livers of patients with a variety of chronic liver diseases, primarily in inflammatory areas in which an intense remodeling is occurring.[117] Second, this crosslinking ability of TG2 appears to have a crucial role in the fixation and activation of TGF-β,[57] the most fibrogenic cytokine.[130] TGF-β1 is released in a latent form (~300 kD) and converted to an active form of 25 kD. Enhanced TG2 activity is required in many tissues for this activation of TGF-β via its crosslinking of large latent complexes to the cell surface or to FN and other ECM components through a latent TGF-β-binding protein portion.[57,61,62,131,132] In addition to the liver, similar fibrogenic roles via the generation of TGF-β have been reported in articular cartilage,[60] kidney,[61] lung,[59] and pancreas.[133]

Third, in *in vivo* models of hepatic apoptosis and in ASH patients, Sp1 is crosslinked, oligomerized, and inactivated by nuclear TG2, leading to the activation of a caspase-independent apoptotic process resulting from the reduced expression of *c-Met*.[12] The TG2-induced decrease in *c-Met* may be involved in the impaired hepatocyte regeneration observed in patients with alcoholic liver diseases.[13,134,135] Furthermore, Giebeler *et al.*[136] reported that the downregulation of *c-Met* is associated with liver fibrosis, indicating a novel apoptotic axis accompanied by liver fibrosis, namely, a nuclear TG2/crosslinked Sp1/decline in *c-Met*. Supporting this hypothesis, the nuclear accumulation of TG2 and crosslinking of Sp1 are observed in the fibrotic area of patients with ASH.[137]

In summary, increased TG2 activity is associated with ECM production and TGF-β, as shown after chronic CCl_4 intoxication in rats[118] directly by stabilizing the ECM in an insoluble form and indirectly by promoting the generation of active TGF-β, which strongly enhances hepatic fibrogenesis by increasing ECM production. The reaction is Ca^{2+} dependent and is classically reported to be the biochemical basis for TG2 involvement in hepatic fibrosis.[129] Therefore, amine substrates, such as putrescine and cystamine (competitive inhibitors of the crosslinking activity of the enzyme), have been shown to be protective in ethanol-induced liver injury as well as liver fibrosis induced by CCl_4.[138–140]

In contrast, Popov *et al.*[141] demonstrated no change in the extent of liver fibrosis after the treatment of $TG2^{-/-}$ mice with CCl_4 or thioacetamide. Again, potential explanations for this discrepancy include differences in mouse background, the method used for *TG2* gene targeting, and broad biological and non-specific activities of TG inhibitors.

Anti- *Versus* Pro-Apoptotic Roles of TG2 in Cancer Cells Partially Through the Regulation of Rb and E2F1 Activities

Multiple studies have shown elevated TG2 expression in many types of cancer cells[9] to be associated with increased drug resistance, metastasis, and poor patient survival (Table 4).[33,142] For example, an analysis of more than 30,000 genes from tumor samples revealed that *TG2* is a highly expressed gene in pancreatic adenocarcinoma.[143] An important property of the highly malignant tumor cells is their ability to survive in hostile host environments as they passes through the lymphatic system or the bloodstream in their attempt to colonize distant sites. TG2 stabilized contact points of tumor cells with the subendothelial matrix in free-floating melanoma cells isolated from arterioles.[144] In contrast, the downregulation of TG2 expression in melanoma cancer cells promoted their ability to metastasize.[145] The ectopic expression of TG2 in a highly malignant hamster fibrosarcoma cell line significantly reduces tumor incidence despite the fact that TG2-transfected clones exhibit no significant differences in growth rates, cell morphology, or levels of spontaneous apoptosis *in vitro*.[146] This finding indicates a suppressive effect of TG2 on tumor growth and confirms the importance of TG2 in the phenotypic changes associated with cancer. Similar nuclear TG2-mediated cell death (as observed in ASH and NASH) has been found in the chemoprevention of cancer. Acyclic retinoid, a synthetic retinoid, stimulates the nuclear localization and activation of TG2,[147] resulting in the crosslinking and inactivation of Sp1, thereby causing cell death in hepatocellular carcinoma cell lines through the downregulation of EGF receptors.[75] In contrast, TG2 enhances EGF receptor expression in glioblastomas and then induces cell transformation.[148] Therefore, a precise understanding of the expression and function of TG2 in the context of cancer stages and types is important for the implementation of TG2-based interventions to disrupt malignant invasion, growth, and survival.

In U937 human leukemic monocyte lymphoma cells undergoing apoptosis, nuclear TG2 polymerizes Rb protein, which culminates in the loss of anti-apoptotic action by Rb protein due to its interaction with E2F1 and the prevention of Rb degradation, which accelerates the degradation of E2F1 and leads to cell growth arrest/apoptosis.[149] In contrast, in some cell lines (NIH3T3 mouse embryonic fibroblast cells, HL-60 human promyelocytic leukemia cells, and mouse embryonic lung fibroblasts) treated with RA or *N*-(4-hydroxyphenyl) retinamide, TG2 prolongs the anti-apoptotic action of Rb protein,[150] protecting it from degradation by caspase-7 due to transamidation and/or the GTP-binding activities instead of undergoing polymerization. This conclusion has been drawn from the finding that monodansylcadaverine, a transamidation inhibitor, blocks the protective effect, whereas a transamidation-defective [C277V] TG2 mutant also exerts a protective effect.[150] The latter is corroborated in HEK293 human embryonic kidney cells in which nuclear localization of the [C277S] TG2 mutant attenuates apoptosis due to its GTP binding or scaffold protein characteristics upon complexing with Rb protein.[151] Furthermore, the phosphorylation of the Rb protein at Ser780 by TG2 destabilizes the Rb-E2F1 complex, ameliorating apoptosis in MCF-7 human breast carcinoma cells, which is further stimulated by the phosphorylation of TG2 with protein kinase A and abrogated by high Ca^{2+} concentrations.[37] These observations indicate that in general, although the outcome differs depending on cell types and treatments, nuclear TG2 heavily polymerizes Rb protein under

extremely high Ca^{2+} concentrations, and low ATP concentrations, resulting in apoptosis through accelerating E2F1 degradation; otherwise, nuclear TG2 stabilizes Rb protein through the phosphorylation of Ser780 and the transamidation of certain glutamine residue(s) (but not through crosslinking) and preventing Rb degradation as a scaffold protein. This behavior indicates that intranuclear Ca^{2+} and GTP concentrations are important in determining whether TG2 acts as Dr. Jekyll or Mr. Hyde (opposing functions).[16]

Diabetes

TG2 is involved in the membrane-mediated events required for glucose-stimulated insulin release from pancreatic β cells (Table 5).[152–154] Vitamin A also induces insulin secretion from islets via TG2,[155] and steroid hormone-induced TG2 facilitates the crosslinking of IGFBP1 and increases IGF-I actions.[156] As a controversial effect, inhibitors of TG2, such as monodansylcadaverine and glycine methylester, do not prevent insulin secretion induced by either cAMP or phorbol ester at basal levels (10 nM) of Ca^{2+}; however, these inhibitors prevent insulin secretion induced by Ca^{2+}.[157] These data indicate that the transamidation activity of TG2 has a critical role in insulin secretion.

$TG2^{-/-}$ mice have impaired glucose-stimulated insulin secretion and show glucose intolerance after intraperitoneal glucose loading.[158] The $TG2^{-/-}$ mouse phenotype resembles that of maturity-onset diabetes in young patients who have several missense mutations located near the catalytic site of TG2 and impaired transamidation activity.[158,159] However, TG2-disrupted mice and the constitutive transamidation active form of TG2-expressing mice show no significant differences in responses to a glucose or insulin challenge, suggesting that glucose homeostasis is TG2 independent.[160]

Blood Vessel Formation

TG2 has a positive role in the formation and stability of blood vessels (Table 6).[161,162] Coeliac disease-specific autoantibodies targeting TG2 disturb several steps of angiogenesis, including endothelial sprouting, the migration of both endothelial, and vascular mesenchymal cells, ECM degradation, the organization of the actin cytoskeleton in capillary cell types, alterations of cell-ECM interactions to thereby affect endothelial cell adhesion, polarization, and motility.[163–165] At the endothelium, TG2 co-localizes with integrin and has an important role in cell spreading and adhesion.[113,166] Moreover, TG2 is co-localized with endostatin in the ECM secreted by endothelial cells under hypoxia, which stimulates angiogenesis and tumorigenesis.[167,168] TG2 inhibition leads to a reduction in FN deposition and matrix-bound VEGFA in HUVECs; TG2 thus positively regulates angiogenesis in a VEGF-dependent manner.[169] Microarray data show that TG2 is specifically upregulated by turbulent shear stress in coronary artery endothelial cells.[170] Given the important role of shear stress in the development of atherosclerosis, we assert that TG2 relates the progression of this disease. In an opposite effect, matrix changes induced by TG2 lead to the inhibition of angiogenesis and tumor growth,[171] indicating that endothelial TG2 primarily promotes blood vessel formation via enhanced stabilization and migration of neovascularization; however, a part of extracellular TG2 reduces organized vasculature via the excess stabilization and accumulation of ECM following their crosslinking. In cultured endothelial cells, TG2 suppressed cell migration via the formation of TGF-β.[57] In vascular smooth muscle cells, TG2 is involved in the all-*trans* RA-mediated apoptosis and reduction of neointimal mass in balloon-injured blood vessels.[54] More recently, TG2 was shown to block extracellular VEGF's binding to heparan sulfate proteoglycans and inhibit an early stage of angiogenesis.[172]

Thus, the effect of TG2 on angiogenesis appears to differ depending on its stage and TG2's subcellular localization.

Conclusions and Prospects

The crosslinking activity of TG2 appears to represent the dominant function of TG2 during apoptotic cell death accompanied by an elevation of intracellular Ca^{2+}. In addition to crosslinking activity, at normal intracellular Ca^{2+} concentrations, the diverse cellular functions of TG2 appear to be attributed to G_h, PDI, and kinase activities. These various biochemical activities of TG2 are differentially regulated depending on its subcellular localization; they also determine whether TG2 has Dr. Jekyll or Mr. Hyde. In the outer membrane, TG2 is released from cells by an unknown mechanism and constitutively exhibits crosslinking activity for ECM and anchoring protein. In the intracellular membrane and cytosol, TG2 typically adopts a closed conformation with the binding of GTP but not that of Ca^{2+}, which contributes to intracellular Ca^{2+} homeostasis, cell proliferation, and other actions. Both growth-stimulating (anti-apoptotic) and pro-apoptotic functions of TG2 may depend on the cell type, the type of death stimuli, the intracellular localization of the enzyme and which of its activities are switched on.[15]

Thus, the use of an existing inhibitor, such as cystamine, is not effective for the regulation of TG2-related pathogenesis. It is important to develop specific regulatory compounds against crosslinking, GTPase, FN-binding, PDI, the phosphorylation activities of TG2, and its cofactors. To control cell death and survival, we are now establishing and screening inhibitors that regulate the nuclear localization of TG2.

Conflict of Interest

The authors declare no conflict of interest.

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