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Tissue transglutaminase in fibrosis - more than an ECM crosslinker

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Tissue transglutaminase (TG2) is upregulated in the pathogenesis of a wide variety of chronic diseases. In this review, special emphasis will be placed on fundamental mechanisms underlying the critical role of TG2 in fibroproliferative disorders. TG2 is best known for its cross-linking capacities in the extracellular space but has many critical and multifaceted roles beyond protein cross-linking, which are driven by the conformation and specific localization of the molecule. As extracellular cross-linker TG2 promotes fibrotic disease through the storage of latent transforming growth factor (TGF)-β1 in a stiffened extracellular matrix. As membrane-bound cell adhesion cofactor and signaling protein and intracellular cross-linker or G-protein, TG2 promotes fibrotic disease through cell survival and profibrotic pathway activation on a signaling, transcriptional, and translational level. Similarities between the roles that TG2 plays in scar tissue and in the tumor stroma suggest that a deeper understanding of key common pathways in disease pathogenesis and progression might lead to the identification of novel treatment targets and the development of new drugs and diagnostic methods.

Tissue transglutaminase (TG2), also named tTG, EC 2.3.2.13, is the most studied representative of a structurally and functionally related family of proteins of which nine members have been identified in humans [1,2]. TG2 is best known for its catalytic transamidation activity, resulting in the Ca2+-dependent post-translational formation of covalent isopeptide bonds between glutamine and lysine residues [1]. Beyond its catalytic core, TG2 consists of an N-terminal β-sandwich and two C-terminal β-barrel domains (Figure 1, left) [3–5]. Far less understood are TG2’s multiple functions at the cell membrane, in the cytoplasm, and in the cell nucleus, such as adhesion, migration, growth, proliferation, survival, apoptosis, differentiation, and phenotype modulation [6,7]. TG2 is an integrin and syndecan-binding adhesion coreceptor for fibronectin (Fn). Approximately 5–40% of β1 integrins are in complex with TG2 and almost all cell-membrane bound TG2 forms 1:1 complexes with integrins [8,9], which suggests a prominent role for TG2 at the cell membrane. While the focus is often on its cross-linking capacities in the extracellular space, TG2 is also active through nonenzymatic protein–protein interactions in both the extra- and intracellular space as further highlighted below. The reader is also referred to the following comprehensive review [10].

TG2 is upregulated in the pathogenesis of a wide variety of chronic diseases
In most cases, the pathophysiological significance of TG2-induced modifications remains unclear. However, it is well documented that TG2 is involved in the pathogenesis of a wide variety of diseases, most notably neoplastic and fibroproliferative, including most malignant cancers and pulmonary, kidney, and cardiac fibrosis [11–14]. TG2 expression is highly correlated with cancer cell survival, malignancy, metastasis, and treatment resistance [13,15]. TG2 morphologically and functionally ‘shapes’ the tumor and scar tissue stroma through extracellular matrix (ECM) cross-linking and binding of transforming growth factor (TGF)-β1 to the ECM, thus priming TGF-β1, one of the most effective profibrotic stimuli, for release and activation [16,17]. Also, through its cell attachment and signaling mediator functions and its intracellular signaling and cross-linking functions, TG2 can promote tumor cell survival and the development of fibrotic (scar) cell phenotypes.
Functional domains, binding sites, and structure of tissue transglutaminase (TG2). Beyond its catalytic core (140–454), TG2 consists of an N-terminal β-sandwich motif (1–139) and two C-terminal β-barrel domains (1: 469–591; 2: 592–687) [3–5]. The most relevant functional binding sites will be described briefly. (a) Recent studies identified residues K30, R116, and H134 on the N-terminal β-sandwich (yellow) as critical binding partners for the 42-kDa gelatin binding domain of Fn [8]. According to Hueltsz-Prince et al. [4], TG2-integrin binding is disrupted upon deletion of C-terminal β-barrel 2 (blue), which indicates that integrin binding sites reside on that domain. The catalytic core region (magenta) contains the active cross-linking site with the catalytic triad residues (C277, H335, D358). GTP (nucleotide) binding takes place on the catalytic core (K173) and β-barrel 1 (green) (R476, R478, V479, M483, R580, Y583) [70]. Heparin binding sites were identified on the catalytic core and β-barrel 2 [71]. (*) Wang et al. [9] identified an alternative heparin binding site. Ca^{2+} binding to five of six Ca^{2+} binding sites on the catalytic core fosters allosteric catalytic site activation [72]. For information regarding further binding sites (α1-adrenergic receptor, PLCδ1, nuclear export signaling peptide), the reader is referred to the following references [70,71]. (b) X-ray crystallography demonstrated that enzymatic cross-linking activity of TG2 is only possible in the Ca^{2+}-induced open-state conformation (PDB: 2Q3Z, [3] with exposed catalytic triad residues blocked by β-barrels 1 and 2 [73]. However, because TG2 has many nonenzymatic functions, ‘functional state’ and ‘activity’ must be carefully distinguished [24]. Figures are adapted and modified with permission from A [70] and B [24].

Major attempts are thus underway to develop potent TG2 inhibitors.

Environmental sensing through major conformational TG2 changes

The biochemical functions of TG2 largely depend on its molecular conformation (Figure 1). Various environmental factors cause allosteric conformational changes and include extracellular, intracellular, and intranuclear Ca^{2+} concentrations, guanosine diphosphate (GDP) and guanosine triphosphate (GTP) concentrations, and matrix metalloproteinase (MMP)-mediated release of membrane bound TG2 into the ECM [7,9]. When bound to GTP or GDP, TG2 adopts a closed conformation with the two C-terminal β-barrel domains folded in and blocking substrate access to the catalytic site. However, with excess Ca^{2+} levels, TG2s affinity for GDP and GTP is reduced, leading to a very large change in molecular shape presenting an open molecular conformation with an accessible active catalytic cross-linking site (Figure 1b) [3,24]. Low Ca^{2+} and high GDP/GTP concentrations in the cytoplasm cause TG2 to adopt a predominantly closed conformation inside the cell [25]. The conformation that TG2 adopts at the cell membrane is largely unknown. No structure has yet been solved of TG2 complexed to any binding partner even though Fn can bind to the open and closed conformations of TG2 [5]. Closed state cytosolic TG2 can bind to heparan sulfate epitopes on exosome membrane–associated syndecan-4 molecules which have a high binding affinity for the closed conformation of TG2. Syndecan-4–dependent translocation of TG2 from the cytoplasm to the extracellular space takes place by fusion of exosomes with the outer cell membrane [9,26–28]. This mechanism was identified in fibrotic kidney disease by Furini et al. [27]. Syndecan-4–associated TG2 delivered to the cell surface can interact with integrins and fibronectin to form membrane-associated protein complexes exerting cell adhesion/receptor-protein functions (Figures 2 and 3) [7,29]. MMP2/9 activity can lead to syndecan-TG2 shedding from the cell surface (Figure 3) [9]. Finally, TG2 predominantly adopts an open conformation in the extracellular space because of high Ca^{2+} and low GDP/GTP concentrations [24]. Formation of two disulfide bonds between cysteine residues under oxidizing conditions at the catalytic site renders TG2 catalytically inactive in its open conformation [30]. Active oxygen reduction mechanisms in the ECM, therefore, promote the catalytic ECM cross-linking activity of TG2 in the extracellular space [31]. Mechanical forces within the physiological range for...
TG2 functions depend on its localization, as well as its allosterically regulated conformation as upregulated in many diseases. Resident fibroblasts deposit and remodel the ECM in healthy tissues (top and middle left) [44], whereas α-SMA expressing contractile myofibroblasts are the key players in fibrotic disease (top and middle right). Myofibroblast-dominated tissues are characterized by increased ECM fiber density and cross-linking. TG2 and TGF-β1 are both upregulated in fibrotic tissues. The open conformation of TG2 cross-links ECM fibrils [1] and binds latent TGF-β1 via LTBP-1 to the ECM [1,34,74]. TG2 interacts in closed or unknown conformation with cell membrane proteins [9,29,41]. Cytosolic and nuclear TG2 activate intracellular signaling pathways and downstream gene expression through enzymatic (open state TG2) and nonenzymatic (closed state TG2) mechanisms. ECM, extracellular matrix; α-SMA, alpha smooth muscle actin; TG2, transglutaminase.
Therefore, it is likely that cells that generate mechanical forces and pull at adhesion sites, which are physically connected to the extracellular matrix, can induce TG2 ‘opening’ and catalyze disulfide bond reduction, thus inducing and stabilizing an open catalytically active conformation of TG2 [3,4].
**Extracellular and intracellular: transamidation and cross-linking functions of TG2/TGF-β1 storage in the ECM.**

The catalytic activity of TG2 can affect protein conformation by generating intramolecular cross-links and can catalyze the formation of covalently linked dimers, oligomers, and polymers [6]. The transamidation activity of TG2 results in protease-resistant intramolecular or intramolecular isopeptide bonds which effectively cross-link ECM fibrils, stiffening the ECM and protecting the intramolecular isopeptide bonds which effectively cross-link ECM fibrils, stiffening the ECM and protecting the ECM from proteolytic degradation [24]. A matrix reservoir of inactive TGF-β1 is formed via TG2-mediated cross-linking of latent TGF-β1 binding protein (LTBP-1) to the ECM (Figures 2 and 3) [33,34]. ECM-bound TGF-β1 can be activated via mechanical release (Figure 3), which is especially effective when the LTBP-1 is bound to a stiff, deformation-resistant ECM.

**Cell membrane: TG2 as signaling protein**

Almost all cell membrane-associated TG2 is bound to integrins [8], where TG2 dimers facilitate integrin cluster formation (Figure 3) [7,19]. Further ECM components (e.g. Fn) and cell membrane protein receptors that bind next to fibronectin's RGD-specific integrin binding site (e.g. Syndecan-4, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and epidermal growth factor (EGF) receptors) can be engaged by these TG2-integrin complexes to execute various cell adhesion (described previously) and signaling functions (Figure 3) [7,9,21,29]. TG2-integrin clusters, TG2-Fn-integrin, and TG2-Fn-integrin-syndecan-4 complexes are known to activate well-reviewed integrin signaling cascades, resulting in RhoA-Rho-associated protein kinase (ROCK) activation, actin filamentation, and downstream release and nuclear translocation of myocardin-related transcription factor A (MRTF-A) and yes-associated protein (YAP)/transcriptional coactivator with PDZ-binding motif (TAZ) (Figure 3) [7,8,19,46,47]. Nuclear MRTF-A and YAP/TAZ both act as transcription cofactors that promote expression of profibrotic genes, including alpha smooth muscle actin (α-SMA), tenascin-C, and connective tissue growth factor (CTGF), which can influence the tumor stroma and cancer development and promote fibrotic disease [17,48,49].

**Inside the cell: enzymatic and nonenzymatic TG2 activity**

With low Ca²⁺ and high GDP/GTP concentrations in the cytoplasm, TG2 binds to and hydrolyzes GTP, assumes a closed conformation, and acts as a G protein. For example, TG2 binds to c-Src and PI3-kinase, facilitating c-Src-dependent phosphorylation of PI3-kinase which promotes cell survival [22]. Owing to fluctuations in Ca²⁺ levels, cytoplasmic open conformation cross-linking activity of TG2 can also be observed with various specific and unspecific target proteins. For example, TG2 drives the constitutive activation of NFκB through its cross-linking activity, which leads to...
increased TGF-β1 expression (e.g. fibrotic diseases, cancer stroma), promotion of epithelial-mesenchymal transformation (increasing tumor malignancy), and cell survival [23,49]. Similarly, RhoA cross-linking and activation leads to increased stress fiber formation [50] and certain translation regulatory proteins like YB-1 can be cross-linked in a state that drives increased α-SMA protein translation [20]. TG2 also regulates mitochondrial function and can initiate mitochondrial-driven apoptosis by cross-linking mitochondrial proteins in its open, catalytically active state [24,51]. At the same time, TG2 cross-linking stabilizes the structure of dying cells, prevents leakage of proteolytic enzymes, and protects the environment of the cell from further damage [52]. Finally, high TG2 levels increase auto- phagy of the tumor suppressor p53, which can help tumor cells escape apoptosis [53].

Nuclear TG2 has been shown to regulate gene expression via post-translational modification of transcriptional factors and related proteins, including Sp1, hypoxia-inducible factor 1 and histones [54]. The G-protein function of cytosolic TG2 typically enhances cell survival [22], whereas the transamidation activity of TG2 can lead to cell death or survival or even fibrotic changes [20,23,49–51,55], depending on the target protein(s). Nuclear localization of TG2 is generally protective against cell death [56,57].

**TG2 as major player in the development and maintenance of fibroproliferative diseases**

Fibroproliferative disorders cause approximately 45% of the mortality in the developed world and appear in a wide spectrum from systemic to organ-specific fibrotic diseases. Besides increased mechanical tissue tension and the presence of certain ECM components, such as cellular fibronectin’s ED-A domain, TGF-β1 is one of the most effective profibrotic stimuli that drive the transformation of fibroblasts into myofibroblasts [17]. Myofibroblasts are the key players within fibroproliferative disorders [17,58]. They incorporate an excess of collagen and other fibrous proteins into the ECM while expressing strong contractile α-SMA positive stress fibers which contract the fibrotic matrix into a stiff, dysfunctional scar [17,33,48,59]. Interestingly, TG2 and TGF-β1 reinforce each other in the progressive fibrotic and tumor stroma microenvironment [49]. As described in previous sections, TG2 facilitates the activation of TGF-β1 in the ECM [16], the activation of profibrotic signaling cascades downstream from TGF-β-integrin—containing membrane signaling complexes [7] and the translation of α-SMA [20]. TG2-β1, on the other hand, promotes TG2 transcription [60,61], leading to self-amplification of TG2 and TGF-β1 in fibrotic tissues and explaining why both are upregulated in fibroproliferative disorders. The fact that TG2 knockout mice were protected against fibrosis [62,63] and that TG2 inhibition in mouse models of fibrosis significantly reduced the fibrotic phenotype [11,12,64] further underlines the critical role that TG2 plays in the pathogenesis and maintenance of fibrosis [65]. The relative contributions of catalytic and noncatalytic TG2 functions to healthy and diseased microenvironments still remain unknown. Inhibition of TG2’s catalytic function can reduce cardiac fibrosis *in vivo* [11,64,66]. Wang et al. and Shinde et al. suggested further reduction of fibrosis via noncatalytic TG2-mediated mechanisms: reduced externalization of TG2 as a result of decreased interaction of intracellular TG2 with exosome membrane—associated syndecan-4, decreased TGF-β1-induced myofibroblast formation and reduced activation of fibrosis-associated genes [11,64].

**Outlook: TG2 as common denominator in fibroproliferative and neoplastic diseases**

The consensus in cancer research seems to mirror our aforementioned descriptions of the profibrotic functions of TG2. Extracellular TG2 in its open enzymatically active conformation promotes cancer cell malignancy through cross-linking of the tumor stroma [49,55]. Intracellular closed conformation TG2 promotes cancer cell survival and malignancy through survival pathway and epithelial-to-mesenchymal transition (EMT) activation [22,49]. Stabilizing the open state of TG2 inside the cell promotes cell death and inhibits the malignant phenotype of cancer cells, thus representing a promising new avenue in the treatment of cancer [24,25]. Similarities between scar tissue and tumor stroma [67–69] suggest that the identification of key common pathways involved in the disease pathogenesis and progression might lead to the identification of novel treatment targets and the development of new drugs and diagnostic methods.

Beyond its role as ECM cross-linker, much future research is needed to understand the many roles of TG2, including cell adhesion stabilization and its nuclear functions. Also, translational research is required as only a few TG2 inhibitors are in clinical trials and none is available for clinical use [55].

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**Conflict of interest statement**

Nothing declared.

**References**

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest


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This recent comprehensive review summarizes how chemical and mechanical signals induce the fibroblast-to-myofibroblast transformation and focuses on mechanobiological aspects of the cell-ECM interaction.


A thorough review of the different conformational states of TG2, the binding partners associated with TG2’s different conformational states and the implications in health and disease. The authors elegantly state that the conformation and activity of TG2 must be considered individually. The focus of this review is on novel therapies and cancer.


Stiffness-tuneable substrates for fibroblast cultures were used to demonstrate that high substrate stiffness promotes the co-expression of ED-A fibronectin and LTBP-1. By binding to LTBP-1, the ED-A repeat variant functioned to enhance the storage of latent TGF-β1 in the ECM.


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This excellent review summarizes the current knowledge state regarding LTBP and LTBP related disease. LTBP is a crucial mediator of TGF-β activity and a key substrate of TG2.

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