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MINIREVIEW ARTICLE

TG2, a novel extracellular protein with multiple functions

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Abstract TG2 is multifunctional enzyme which can be secreted to the cell surface by an unknown mechanism where its Ca^{2+}-dependent transamidase activity is implicated in a number of events important to cell behaviour. However, this activity may only be transient due to the oxidation of the enzyme in the extracellular environment including its reaction with NO probably accounting for its many other roles, which are transamidation independent. In this review, we discuss the novel roles of TG2 at the cell surface and in the ECM acting either as a transamidating enzyme or as an extracellular scaffold protein involved in cell adhesion. Such roles include its ability to act as an FN co-receptor for β integrins or in a heterocomplex with FN interacting with the cell surface heparan sulphate proteoglycan syndecan-4 leading to activation of PKCα. These different properties of TG2 involve this protein in various physiological processes, which if not regulated appropriately can also lead to its involvement in a number of diseases. These include metastatic cancer, tissue fibrosis and coeliac disease, thus increasing its attractiveness as both a therapeutic target and diagnostic marker.

Keywords Tissue transglutaminase · Extracellular matrix · Cell adhesion and crosslinking

Abbreviations

TG2	Tissue transglutaminase
TGs	Transglutaminases
PDI	Protein disulphide isomerase
TGFβ1	Transforming growth factor β1
PLC	Phospholipase C
FN	Fibronectin
ECM	Extracellular matrix
HOB	Human osteoblasts
HSPGs	Heparan sulphate proteoglycans
ERK1/2	Extracellular signal-regulated kinase1/2
FITC	Fluorescein isothiocyanate
FAK	Focal adhesion kinase
HUVEC	Human umbilical vein endothelial cells
siRNA	Small interfering RNA
IL	Interleukin
NF-κB	Nuclear factor κB
TNF	Tumour necrosis factor
TGM2	TG2 gene
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
PDGFR	Platelet-derived growth factor receptor
GPR56	G-protein coupled receptor 56
MMP	Matrix metalloproteinase
MT1-MMP	Membrane type1 metalloproteinase
HFDF	Human foreskin dermal fibroblasts
vSMC	Vascular smooth muscle cells
OPN	Osteopontin
BSP	Bone sialoprotein
SPD	Spermidine

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Background introduction

TG family

The posttranslational modification of proteins plays an important role in regulating protein function.

Transglutaminases (TGs) were first reported in 1959 by Mycek and colleagues (Mycek et al. 1959) as an enzyme found in guinea pig liver, able to modify proteins by mediating an acyl-transfer reaction between the γ-carboxamide group of peptide-bound glutamine and a primary amine. The result of this reaction is either protein crosslinking if the amine is the ε-amino group of peptide-bound lysine or modification of the peptide glutamine by crosslinking to a primary amine such as a polyamine. Under slightly acidic conditions in the absence of a suitable primary amine, the deamidation of the peptide-bound glutamine can also occur. This latter reaction by tissue transglutaminase (TG2) has become highly significant in the pathogenesis of coeliac disease (Sollid and Khosla 2005). Because of their ability to crosslink proteins, often resulting in high Mr polymers, TGs have been referred to as "Nature's Biological Glues" (Griffin et al. 2002). TGs are found widely in nature, but in mammals their enzymatic activity is Ca^{2+}-dependent, and other factors including GTP/GDP can also affect the activity of some of the mammalian TGs (Verderio et al. 2004). Even though the function of TGs has been under investigation by scientists for more than five decades, not all of the eight members of the mammalian TG family have been fully characterized (Collighan and Griffin 2009). Moreover, further novel enzymatic activities of the ubiquitous TG2 have recently been reported, e.g. the protein disulphide isomerise (PDI) (Hasegawa et al. 2003) and protein kinase activities (Mishra and Murphy 2004), thus further extending the potential physiological and pathological importance of this diverse group of enzymes.

Structure and regulation of TG2

TG2, commonly referred to as "tissue transglutaminase", is probably the most ubiquitous and multifunctional member of the TG family, to which this review is devoted. Since its discovery in 1959, diverse roles have been ascribed to it, but many of its suggested physiological functions still remain an enigma. TG2 is a monomeric protein of 687 amino acids composed of four domains namely the N-terminal β-Sandwich (residues 1–139), the α/β catalytic core (residues 140–454) and two C-terminal β-barrel domains (residues 479–585 and 586–687, respectively) (Fig. 1).

Although found with high expression in a number of mammalian tissues such as in endothelial cells, red blood cells and smooth muscle cells, TG2 is often regarded as a stress-related protein, with its expression upregulated by a number of physiological and pathological stimuli. Of these, retinoic acid (RA) is a well-known inducer for TG2 expression at both mRNA and protein levels, via which RA promotes the expression of TG2 through interaction with its receptors to promote the formation of RAR/RXR heterodimers or RXR/RXR homodimers, which trigger transcription of TGM2 (Glass 1994; Mehta et al. 1996). A number of inflammatory cytokines and growth factors can also induce TG2 expression, including transforming growth factor β1 (TGFβ1) (Quan et al. 2005), interleukins (e.g. IL-6) (Suto et al. 1993) and tumour necrosis factor α (TNFα) (Kuncio et al. 1996), which is thought to upregulate TG2 via activation of the nuclear factor κB (NF-κB).

Cellular location and activity of TG2

Reputed as a protein with diverse functions, it is important to indicate that these function(s) of TG2 are closely related to its cellular or subcellular location under different physiological and pathological conditions.

Originally regarded as an intracellular protein only found in the cytosol but later found in the plasma membrane and subsequently in the nucleus and mitochondria, it is thought that its intracellular Ca^{2+}-dependent transamidating [K_m for Ca^{2+} of 2–3 μM (Hand et al. 1985)] activity is normally silent following the discovery that GTP and GDP act as a negative regulator (Smethurst and Griffin 1996). However under stress, disturbance of the intracellular Ca^{2+} balance may cause transient loss of Ca^{2+} homoeostasis, which could feasibly activate the enzyme leading to induction of the pro-apoptotic and apoptotic functions of the protein. Such a function may occur in the nucleus where the specific crosslinking of the transcription factor SP1 leads to the induction of apoptosis (Kiraly et al. 2009; Tatsukawa et al. 2009). Inside cells, TG2 can act as a Ca^{2+}-independent GTPase and ATPase and has also been reported to be a Ca^{2+}-independent threonine protein kinase (Mishra and Murphy 2006). As a G-protein, TG2 (Gh) is involved in the activation of phospholipase C (PLC) during hormonal signal transduction (Stephens et al. 2004). Mg^{2+}-ATP binding to TG2 inhibits GTPase activity, but does not interfere with the transamidating activity, while Mg^{2+}-GTP binding induces a conformational change that inhibits transamidating activity, but is reported not to affect the ATPase activity of TG2 (Lai et al. 1998) although both nucleotides bind to similar binding pocket (Han et al. 2010). As a protein kinase, TG2 is reputed to regulate the phosphorylation of growth factor-binding protein-3 (IGFBP-3) (Mishra and Murphy 2004). There are still conflicting ideas regarding the presence of TG2 in mitochondria. Recent evidence suggests that mitochondrial TG2 acts as a Ca^{2+}-independent protein disulphide isomerase (PDI) (Hasegawa et al. 2003). There is also accumulating evidence that TG2 can translocate to the nucleus with the help of importin-α-3 protein where it may have a cell survival role by affecting transcription of key genes

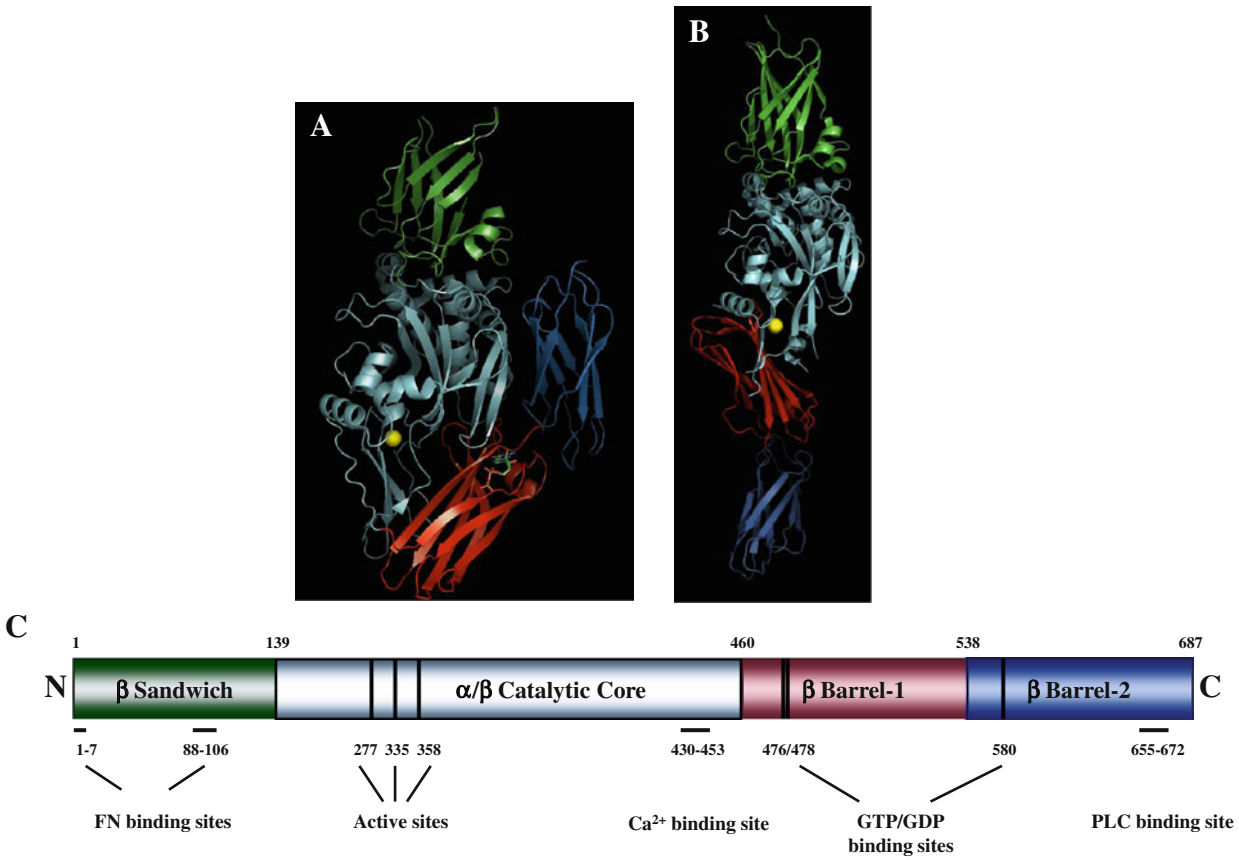

Fig. 1 The structure of TG2 **a** and **b**, molecular structure of TG2 in its closed conformation (**a**) when binding to GTP with the Ca^{2+} binding site shown as the *yellow ball*. **b** The extended form of the enzyme with Ca^{2+} bound at the site of the *yellow ball*. **c** Different domains of TG2 with colours coded to the structures shown in **a** and **b** with the known binding sites denoted

(Peng et al. 1999). It has been reported that intracellular TG2 can bind to the cytoplasmatic domain of α integrins, leading to the inhibition of cell migration (Kang et al. 2004). More recently, intracellular TG2 has also been shown to act as a non-transamidating scaffold protein important in regulating actin cytoskeleton organization (Antonyak et al. 2009).

Secretion of TG2

Unlike classic secretory proteins, TG2 lacks the hydrophobic leader sequences; hence, it cannot be externalized via a conventional endoplasmic reticulum/Golgi-dependent pathway (Verderio et al. 2004). Indeed, the consensus for a number of years was that TG2 could not be secreted by healthy cells under normal physiological conditions, unless the cells are under stress or damaged leading to leakage of the enzyme into the extracellular matrix (ECM). By putting TG2 expression under the control of a tet-inducible promoter and immunostaining extracellular TG2 prior to its fixation, Griffin and colleagues demonstrated, for the first time, that increased expression of TG2 in 3T3 fibroblasts led to its increased externalization and presence within the ECM. Importantly, this occurred without the need for exogenous stimuli or induction of cell death (Verderio et al. 1998). These studies showed that the enzyme colocalized with fibronectin (FN) (Gaudry et al. 1999a) and β1 integrin (Gaudry et al. 1999b). Subsequent data using FITC-cadaverine incorporation into healthy 3T3 fibroblasts demonstrated TG activity both at the cell surface and in the ECM following increased induction of its expression (Verderio et al. 1998). However, the mechanism by which TG2 is secreted still remains a mystery, although several mechanisms have been proposed and some clues may be gained from the literature. Early studies indicated the requirement for intact N-terminal β-sandwich domain and an intact transamidation active site (Balklava et al. 2002; Gaudry et al. 1999a). Several mutations that affect the conformation of TG2 also affect secretion. Mutation K173L, which abolishes GTP binding, prevents secretion and C277S, which blocks transglutaminase activity, prevents deposition into the extracellular matrix but is found at the cell surface (Balklava et al. 2002; Johnson and Terkeltaub 2005). Both mutations, however, may be expected to hold TG2 into its open conformation. Mutation Y274A, which is thought to prevent the transition between open and closed

conformations, also prevents secretion (Balklava et al. 2002; Johnson and Terkeltaub 2005), suggesting that an ability to alter conformation may be critical for full secretion. There is also evidence to suggest that TG2 secretion may be associated with Integrin β1-rich recycling endosomes (Zemskov et al. 2011), while recent evidence suggests that binding to cell surface heparan sulphate proteoglycans is a requirement for translocation of TG2 onto the cell surface and for subsequent transamidating activity (Scarpellini et al. 2009).

Extracellular TG2

Once secreted into the extracellular environment, TG2 may influence cell behaviour through its Ca^{2+}-dependent transamidating activity, since both high Ca^{2+} and low GTP levels will promote activation of the enzyme if the appropriate substrates are available (see "TG2 a FN coreceptor for integrin mediated cell adhesion" below). However, activation of the enzyme may only be transient due to oxidation of TG2 in the non-reducing extracellular environment leading to the formation of the vicinal disulphide bond between Cys^{370} and Cys^{371} thought to be promoted by Cys^{230} which can also bond with Cys^{370} (Stamnaes et al. 2010). Each of these Cys residues are in close proximity to one another in the crystal structure, both in the open and closed conformations. Nitrosylation of TG2 by its reaction with NO may also play a key role in regulating the switch between an active and inactive transamidating enzyme. Such inactivating mechanisms may be one of the explanations for the ability of TG2 to undertake many other extracellular roles in which transamidation activity is not required (see "TG2 and its interaction with heparan sulphate proteoglycans").

TG2: a novel extracellular scaffold protein

TG2 and its interaction with heparan sulphate proteoglycans

TG2 has high affinities for both fibronectin and heparan sulphate proteoglycans (HSPGs) (19.47 ± 6.92 and 15.92 ± 0.96 nM, respectively) and it has been suggested that HSPGs may be important in the regulation of the translocation of TG2 at the cell surface (Scarpellini et al. 2009). Once externalized and deposited into the ECM, TG2 may act as a novel structural protein when bound to FN in a heterocomplex (TG–FN) (Verderio et al. 1998). The importance of TG–FN as a novel cell adhesion complex compared to FN alone was first shown in human osteoblast (HOB) cells when plated onto TG–FN coated polycaprolactone (Heath et al. 2002) as a means of stabilizing cell surface/biomaterial interfaces. Subsequent studies showed that FN-bound TG2 could also compensate the RGD peptide-induced anoikis (apoptosis caused by the loss of cell adhesion) (Fig. 2a, b), suggesting that the cell adhesion mechanism was independent of integrin-mediated outside-in signalling and therefore distinct from that shown for its interaction with integrins (Verderio et al. 2003). This process was shown to be both protein kinase α (PKCα)- and focal adhesion kinase (FAK)-dependent. Further studies showed that TG2, when bound to FN, acts as a bridging protein by binding to and activating syndecan-4. Following its direct interaction with syndecan-4, the activation of PKCα occurs, which after translocation to the cell membrane binds to the intracellular domain of β1 integrin, triggering inside-out signalling of these integrins via activation of FAK and extracellular signal-regulated kinase 1/2 (ERK1/2) (Telci et al. 2008). More recent work has demonstrated the involvement of another HSPG family member—syndecan-2, which acts as an associate receptor for syndecan-4 through crosstalk via PKCα, since no direct interaction between TG2 and syndecan-2 is found. While acting as a novel scaffold protein through the bridging of syndecan-4 with FN, this complex can also facilitate the enhancement of FN fibril formation mediated through a syndecan-4/2 and $\alpha5\beta1$ integrin co-signalling pathway, which is independent of the requirement for transamidating activity and occurs even when integrin binding sites for FN are blocked by RGD peptides (Fig. 2a, b; Wang et al. 2010). The importance of such a process may explain why delayed wound healing is found in both TG2 knockout and syndecan-4 knockout animals. The same signalling and matrix deposition pathway is also likely to be involved in human osteoblasts during matrix turnover, suggesting the existence of a universal signalling pathway involving syndecan-4/2 and $\alpha5\beta1$ integrin and regulated by the TG–FN heterocomplex (Wang et al. 2011).

TG2—a FN co-receptor for integrin-mediated cell adhesion

TG2 and its high-affinity binding partner FN may also cooperate in promoting activation of integrin signalling pathways important in cell adhesion in a non-transamidating manner (Collighan and Griffin 2009). Given that the TG2 binding site (42 kD gelatin-binding domain) on FN (Lorand et al. 1993) and the integrin binding site (named the RGD cell-binding site) are located in different parts of the FN molecule, it suggests that TG2 and integrins cooperate rather than compete with each other in the cell adhesion process, as such TG2 is thought to act as an integrin-binding adhesion co-receptor for fibronectin (Fig. 2c). Moreover, the binding of cell surface TG2 with FN was shown to activate FAK and increase the GTP loading of the small GTPase RhoA, triggering intracellular

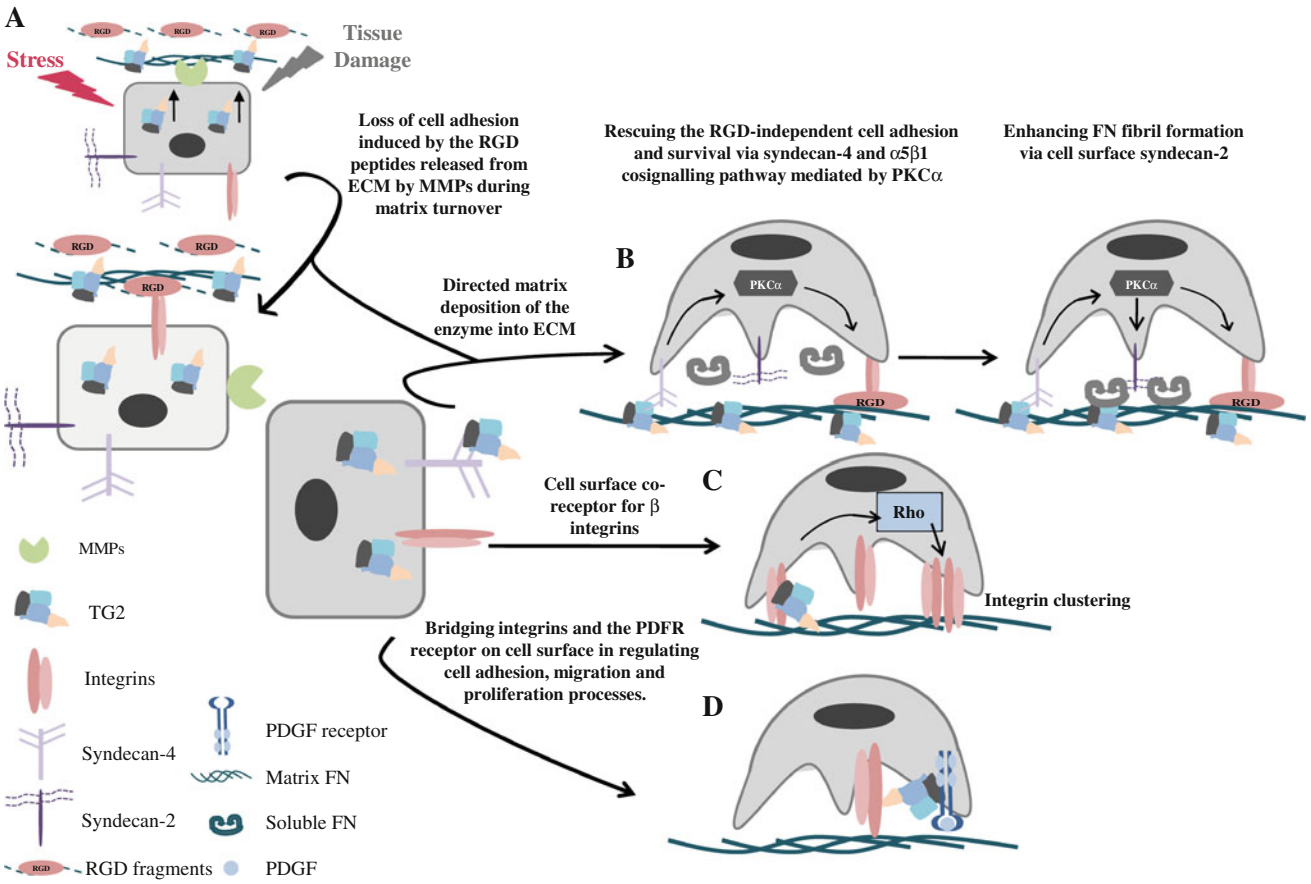

Fig. 2 TG2, a novel extracellular scaffold protein involved in cell adhesion. **a** Following stress or tissue damage, the upregulation of TG2 leads to its externalization into extracellular environment and its deposition into the ECM leading to the formation of TG–FN complexes. Also activated by the stress stimuli, cell surface MMPs can digest the matrix proteins, resulting in the release of the RGD fragments which can block the integrin-mediated cell adhesion on FN. **b** Matrix-bound TG2 can compensate the RGD-independent loss of cell adhesion and restore FN fibril formation and thereby rescue the cells from anoikis (a type of apoptosis induced by the loss of cell adhesion) via syndecan-4/2 and $\alpha 5\beta 1$ integrin co-signalling pathway. **c** TG2 on the cell surface functions as an integrin co-receptor in mediating integrin-related cell adhesion on FN. **d** Cell surface TG2 promotes cell migration and proliferation by acting as a bridging protein between PDFGR and integrins to regulate PDGFR signalling pathway

signalling and induction of cell adhesion (Janiak et al. 2006). Using both immunofluorsecent colocalization and immunoprecipitation studies, cell surface TG2 was shown to interact with $\beta 1$ and $\beta 3$ integrin receptors in fibroblasts and monocytes (Akimov and Belkin 2001), while in human breast cancer cells the direct interaction of TG2 with $\beta 1$, $\beta 4$ and $\beta 5$ integrins has been observed, suggesting that TG2 may be important in regulating cancer cell adhesion, invasion and migration (Mangala et al. 2007). Agreeing with earlier reports, Chen and colleagues reported the over-expression of TG2 and FN in a highly invasive A431-III cell line, in which an increased interaction of TG2 with $\beta 1$ and $\beta 3$ integrins was described that could be suppressed by siRNA silencing of TG2 (Chen et al. 2010). Unlike FN, integrins do not appear to serve as enzymatic substrates of TG2 or of other TGs, and the formation of stable non-covalent integrin–TG2 complexes is independent of the transamidating activity of TG2 (Akimov and Belkin 2001; Akimov et al. 2000). Although the binding sites within TG2 for β integrins are still unknown, it has been demonstrated that integrin–TG2 complexes have a 1:1 stoichiometry and it was suggested that all of the TG2 on the cell surface may be bound to integrin receptors in a FN-independent way (Akimov and Belkin 2001; Akimov et al. 2000). A series of studies led by Mehta and colleagues on tumour cells further demonstrated the connection between TG2 and the integrin-relevant downstream signalling molecule—FAK. The culture of TG2-positive breast cancer cells and TG2-transfected fibroblasts on fibronectin-coated surfaces led to the activation of FAK, one of the downstream signalling molecules of integrins (Mehta et al. 2006). In addition, downregulation of TG2 by siRNA attenuated FN-mediated cell attachment (Herman et al. 2006) and FAK phosphorylation and reduced cell survival (Verma et al. 2006). In human umbilical vein endothelial cells (HUVEC), downregulation of TG2 expression by

small interfering RNA (siRNA) led to the loss of cell adhesion in a p-FAK^{397}-dependent manner, which could be partially compensated by exogenous TG2 treatment (Nadalutti et al. 2011). A study performed in TG2−/− macrophages revealed that over-expression of β3 integrin on the cell surface can partially compensate for the absence of TG2 during the apoptotic cell clearance process, which is dependent on the β3 integrin-Src signalling pathway (Toth et al. 2009). Recently, it was reported that $\alpha5\beta1$ integrin was required by the exogenous GTP-bound TG2, either in a soluble or solid phase, to induce hypertrophic differentiation of chondrocytes, which was dependent on the phosphorylation of FAK at Tyr^{125}, while this effect could not be found in the cells treated with GTP-free TG2. GTP-bound TG2, when present in the ECM, was shown to induce hypertrophic differentiation of chondrocytes through a $\alpha5\beta1$ integrin-dependent and FAK-associated cell adhesion processes (Tanaka et al. 2007). Recently, it has also been reported that on the cell surface, TG2 can bridge $\beta1$ integrins with the platelet-derived growth factor receptor (PDGFR). Via acting as a novel scaffold protein (Fig. 2d), TG2 is thought to promote PDGFR clustering and in turn regulate cell migration via a receptor clustering mechanism (Zemskov et al. 2009).

TG2 and GPR56

Apart from integrins and syndecan-4 receptors, another cell surface receptor reported to be involved in TG2-mediated signalling is the G-protein coupled receptor GPR56. It has been reported that the expression of G-protein coupled receptor 56 (GPR56) is reduced in certain types of human cancers such as pancreatic cancer (Huang et al. 2008). First reported by Xu and colleagues, GPR56, is downregulated in highly metastastic cancers and over-expression of GPR56 leads to the inhibition of cancer proliferation and metastasis, which is related to its specific interaction with matrix TG2 (Xu et al. 2006). A recent report demonstrated a correlation between TG2, GPR56 and NF-κB in oesophageal squamous cell carcinoma (ESCC) tissue samples, suggesting the potential involvement of these proteins in ESCC invasion (Kausar et al. 2011).

TG2 and the MMPs

The presence of TG2 at the cell surface also seems to have a relationship with the presence and activation of cell surface metalloproteinase (MMPs), thought to be important in both cell migration and invasion (Collighan and Griffin 2009). The expression of TG2 can affect the expression level of MMPs and their activities, which appears to be cell-type dependent. In U937 and MCF7 cells, the over-expression of TG2 induced by retinoic acid treatment led to the inhibition of matrix metalloproteinase-9 (MMP-9) activity via blocking its transcription, which can be reversed by addition of a TG2 inhibitor (Ahn et al. 2008). In highly invasive A431-III cells, reduced MMP-9 and MMP-1 secretion was also found to correlate with TG2 knockdown by siRNA treatment. In glioma and fibrosarcoma cells, over-expression of membrane type 1-metalloproteinase (MT1-MMP) can increase the degradation of cell surface TG2, which leads to inhibition of cell adhesion mediated by its interaction with integrin receptors and in turn promotes increased cell migration on FN (Belkin et al. 2001). A recent study demonstrated that MT1-MMP can cleave the extracellular TG2 into a smaller fragment of 56 kDa with an ATP binding site. This fragment presents higher ATPase activity and was shown to be important in the osteoblast pro-mineralization process (Nakano et al. 2010).

TG2, an extracellular crosslinking enzyme with physiological and pathological roles

A number of ECM proteins are potential substrates for TG2 crosslinking including fibronectin, vitronectin, osteonectin, osteopontin, laminin, fibrillin, nidogen, collagen I, collagen II and collagen V and XI and many more (Aeschlimann et al. 1992; Chau et al. 2005; Kleman et al. 1995; Mangala and Mehta 2005). However as previously stated, under normal physiological conditions this transamidating activity in the non-reducing extracellular environment may only be transient prior to its oxidation or its binding to its high-affinity binding partners FN and cell surface heparan sulphates, which may both either direct or regulate its transamidating activity (Scarpellini et al. 2009).

It has been shown that crosslinked FN by TG2 shows high molecular rigidity, which in osteoblasts leads to increased adhesion and the clustering of $\beta1$ integrins (Forsprecher et al. 2009). This finding was confirmed by Nelea and colleagues, in which TG2-crosslinked FN showed high stability against aqueous, high ionic and chaotropic agents, and the high rigidity was confirmed by atomic force microscopy (Nelea et al. 2008). In glioblastoma cells with high TG2 expression level (compared to normal brain tissue), matrix FN deposition can be increased, which can be abolished by a site-directed TG2 inhibitor KCC009. Interestingly in this study, KCC009 treatment led to an increased sensitivity of the glioblastoma cells to chemotherapy, due to the disruption of the FN matrix (Yuan et al. 2007). Studies with HUVEC cells have also suggested that TG2 via its transamidating activity may form an intracellular complex with vascular endothelial growth factor receptor 2 (VEGFR-2), which modulates the endothelial cells response to vascular endothelial growth factor (VEGF) (Dardik and Inbal 2006).

Collagen I, another potential TG2 substrate, can also be crosslinked by TG2 leading to high resistance to protease degradation and matrix turnover. Unlike many other non-enzymic crosslinking agents, TG2-crosslinked collagen shows low toxicity to cells and provides the ideal biomaterial for collagen applications in wound healing in soft and hard tissues such as bones. Indeed, in both HOBs and human foreskin dermal fibroblasts (HFDF), TG2-crosslinked collagen enhances cell adhesion, proliferation and differentiation compared to native collagen (Chau et al. 2005). A study on collagen fibril modification by atomic force microscopy indicated a threefold increase in rigidity compared to native collagen. Seeded on TG2-crosslinked collagen, vascular smooth muscle cells (vSMC) show more organized actin cytoskeletal fibres than non-treated collagen, leading to increased cell spreading and proliferation by an integrin-independent mechanism (Spurlin et al. 2009). However, the increased rigidity and resistance to turnover of collagen crosslinked by TG2 can also lead to tissue fibrosis (Huang et al. 2009). Under these pathological conditions, extracellular TG2 remains highly active through its continuous release from traumatised and/or damaged cells thus overwhelming any extracellular regulatory mechanism. The result is a vicious self-propagating cycle where increased TG2 increases the activation of TGFβ1 and/or NFκB, which in turn increase the expression of TG2 and for TGFβ1 the increased expression of matrix proteins, which are then crosslinked and stabilized by TG2. These processes lead to an increase in the accumulation and deposition of matrix proteins leading to fibrosis and scarring (Johnson et al. 2003; Fig. 3). Such a scenario has been observed in a number of fibrotic conditions including diabetic nephropathy (Huang et al. 2010), in kidney scarring (Johnson et al. 2003) and in atherosclerosis (Van Herck et al. 2010). In these circumstances, nitrosylation of the active site thiol and other cysteine residues may be a key factor in both regulating transamidating activity and deposition of TG2 into the ECM (Lai et al. 2001; Telci et al. 2009). A recent report has suggested that loss of extracellular regulation of the enzyme via a reduction in NO levels commonly found in damaged tissues such as kidney leads to deregulation of activity, again tipping the balance towards matrix accumulation (Telci et al. 2009). In keeping with this finding, decreased *S*-nitrosylation of TG2 has also been shown to contribute to age-related increases in vascular stiffness (Santhanam

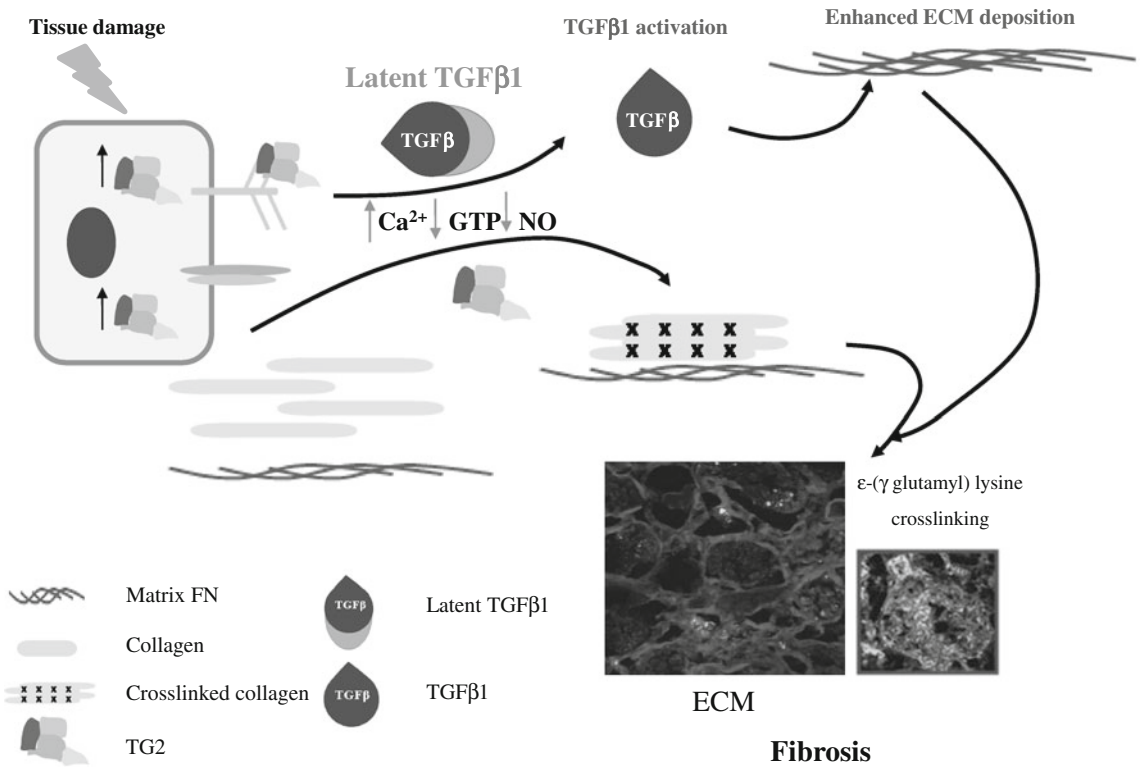

Fig. 3 The importance of TG2 crosslinking activity in the development of fibrosis. Following tissue damage, upregulation of TG2 results in its externalization into the extracellular space where high levels of Ca^{2+}, low levels of GTP and decreasing NO levels lead to activation of the enzyme resulting in the crosslinking of ECM proteins such as collagen, and the activation of TGFβ1. Under normal wound healing, this will lead to ECM remodelling, which in turn can regulate cell adhesion, proliferation and migration. However with continuous insult to the tissue, the regulatory mechanisms controlling TG2 activity fail leading to progressive matrix deposition and crosslinking resulting in the development of fibrosis

et al. 2010). TG2 can also promote the formation of heterocomplexes of ECM proteins. In MC3T3 osteoblasts, TG2 has been proposed to modulate the formation of FN and collagen networks, ECM assembly and osteoblast differentiation and eventually mineralization (Al-Jallad et al. 2006). In another study, a hetero-complex of osteopontin (OPN) and bone sialoprotein (BSP) crosslinked by TG2 showed improved osteoblast adhesion. The active enzyme has also been used to incorporate polyamines, such as spermidine (SPD), into cell basement membrane components including laminin and Matrigel. Once incorporated, these modified matrices significantly reduced cell B16-F10 melanoma cell adhesion, accompanied with reduced MMP-9 activity, suggesting that the modification of basement membrane components by TG2 may be a natural self-defence mechanism against tumour invasion.

Interestingly, a series of studies using CT26 colonal cancer cells involving tumour injection of the active enzyme led to the inhibition in tumour growth and improved animal survival rates, accompanied with increased collagen I deposition in the tumour stroma (Jones et al. 2006). In agreement with this finding, over-expression of the active TG2, but not the inactive enzyme, in CT26 cells led to reduction in tumour growth and an increase in animal survival rate. Increased crosslinked matrix FN deposition was found in the cells transfected with active protein accompanied by a TG2-dependent activation of TGFβ1 (Kotsakis et al. 2010). Contrary to these findings, inhibition of TG2 in highly metastatic and drug-resistant breast cancer, pancreatic ductal adenocarcinoma, lung carcinoma and melanoma cells leads to decreased cell survival. In many cases upregulation of TG2 is generally accompanied by the constitutive activation of NF-κB leading to drug resistance and the facilitation of tumour metastasis. Moreover, the activation of NF-κB is found in many advanced stage tumours. Normally, activation of NF-κB is transient occurring via the conventional pathway involving phosphorylation of the inhibitory protein IκBα, leading to its dissociation from NF-κB and in turn the activation of NF-κB. However, the association of TG2 with NF-κB and/or the crosslinking of IκBα may be an alternative pathway for the activation of NF-κB in advanced tumours where TG2 is found to be upregulated (Mehta et al. 2010). More recent studies have also suggested that in over-expressing breast cancer cells, increased TG2 activity is important in epithelial/mesenchymal transition via a TGFβ1-dependent pathway (Kumar et al. 2010).

Given the increased awareness for the involvement of transglutaminases, particularly TG2, in disease processes, there is now much interest in developing specific drugs that target a particular isoform of this large family of enzymes. Indeed, the search for specific inhibitors of TG2, in particular active site-directed irreversible inhibitors, has been the interest of a number of groups and commercial organisations [see (Siegel and Khosla 2007) for a recent review]. However despite the application and evaluation of these compounds in preclinical studies (Huang et al. 2009; Yuan et al. 2007), very little progress in the clinic has been made. In preclinical studies, irreversible active site-directed inhibitors (Griffin et al. 2008) used in animal models of renal scarring (Johnson et al. 2003) and diabetic nephropathy led to up to 90% reduction in scarring and a parallel reduction in protein crosslinking with a significant improvement in kidney function (Huang et al. 2009). In cancer studies, animal models using subcutaneous transplanted murine DBT glioblastomas treated with dihydroisoxazole-based TG inhibitors plus the chemotherapeutic agent BCNU led to increased tumour cell death (Yuan et al. 2005). In another study, TG2 silencing using siRNA was also demonstrated to be an effective way of treating tumours as demonstrated in mice transplanted with pancreatic ductal adenocarcinoma (Verma et al. 2008).

Conclusion

In this review, we have aimed to demonstrate the important roles that TG2 has in the extracellular environment, where it may act both as a transamidating and non-transamidating protein regulating a number of both physiological and pathological events. However what determines the transition between these different roles of TG2 both at the cell surface and in the ECM, whether it is physiological or pathological, is still the subject of intense research.

References

Aeschlimann D, Paulsson M, Mann K (1992) Identification of Gln (726) in nidogen as the amine acceptor in transglutaminase-catalyzed cross-linking of laminin–nidogen complexes. J Biol Chem 267:11316–11321

Ahn JS, Kim MK, Hahn JH, Park JH, Park KH, Cho BR, Park SB, Kim DJ (2008) Tissue transglutaminase-induced down-regulation of matrix metalloproteinase-9. Biochem Biophys Res Commun 376:743–747

Akimov SS, Belkin AM (2001) Cell surface tissue transglutaminase is involved in adhesion and migration of monocytic cells on fibronectin. Blood 98:1567–1576

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

Al-Jallad HF, Nakano Y, Chen JL Y, McMillan E, Lefebvre C, Kaartinen MT (2006) Transglutaminase activity regulates osteoblast differentiation and matrix mineralization in MOT3–E1 osteoblast cultures. Matrix Biol 25:135–148

Antonyak MA, Li B, Regan AD, Feng Q, Dusaban SS, Cerione RA (2009) Tissue transglutaminase is an essential participant in the epidermal growth factor-stimulated signaling pathway leading to

cancer cell migration and invasion. J Biol Chem 284:17914–17925
Balklava Z, Verderio E, Collighan R, Gross S, Adams J, Griffin M (2002) Analysis of tissue transglutaminase function in the migration of Swiss 3T3 fibroblasts: the active-state conformation of the enzyme does not affect cell motility but is important for its secretion. J Biol Chem 277:16567–16575
Belkin AM, Akimov SS, Zaritskaya LS, Ratnikov BI, Deryugina EI, Strongin AY (2001) Matrix-dependent proteolysis of surface transglutaminase by membrane-type metalloproteinase regulates cancer cell adhesion and locomotion. J Biol Chem 276:18415–18422
Chau DYS, Collighan RJ, Verderio EAM, Addy VL, Griffin M (2005) The cellular response to transglutaminase-cross-linked collagen. Biomaterials 26:6518–6529
Chen SH, Lin CY, Lee LT, Chang GD, Lee PP, Hung CC, Kao WT, Tsai PH, Schally AV, Hwang JJ, Lee MT (2010) Up-regulation of fibronectin and tissue transglutaminase promotes cell invasion involving increased association with integrin and MMP expression in A431 cells. Anticancer Res 30:4177–4186
Collighan RJ, Griffin M (2009) Transglutaminase 2 cross-linking of matrix proteins: biological significance and medical applications. Amino Acids 36:659–670
Dardik R, Inbal A (2006) Complex formation between tissue transglutaminase II (tTG) and vascular endothelial growth factor receptor 2 (VEGFR-2): proposed mechanism for modulation of endothelial cell response to VEGF. Exp Cell Res 312: 2973–2982
Forsprecher J, Wang Z, Nelea V, Kaartinen MT (2009) Enhanced osteoblast adhesion on transglutaminase 2-crosslinked fibronectin. Amino Acids 36:747–753
Gaudry CA, Verderio E, Aeschlimann D, Cox A, Smith C, Griffin M (1999a) Cell surface localization of tissue transglutaminase is dependent on a fibronectin-binding site in its N-terminal beta-sandwich domain. J Biol Chem 274:30707–30714
Gaudry CA, Verderio E, Jones RA, Smith C, Griffin M (1999b) Tissue transglutaminase is an important player at the surface of human endothelial cells: evidence for its externalization and its colocalization with the beta(1) integrin. Exp Cell Res 252:104–113
Glass CK (1994) Differential recognition of target genes by nuclear receptor monomers, dimers, and heterodimers. Endocr Rev 15:391–407
Griffin M, Casadio R, Bergamini CM (2002) Transglutaminases: nature's biological glues. Biochem J 368:377–396
Griffin M, Mongeot A, Collighan R, Saint RE, Jones RA, Coutts IGC, Rathbone DL (2008) Synthesis of potent water-soluble tissue transglutaminase inhibitors. Bioorg Med Chem Lett 18:5559–5562
Han BJ, Cho JW, Cho YD, Jeong CK, Kim SY, Lee BI (2010) Crystal structure of human transglutaminase 2 in complex with adenosine triphosphate. Int J Biol Macromol 47:190–195
Hand D, Bungay PJ, Elliott BM, Griffin M (1985) Activation of transglutaminase at calcium levels consistent with a role for this enzyme as a calcium receptor protein. Biosci Rep 5:1079–1086
Hasegawa G, Suwa M, Ichikawa Y, Ohtsuka T, Kumagai S, Kikuchi M, Sato Y, Saito Y (2003) A novel function of tissue-type transglutaminase: protein disulphide isomerase. Biochem J 373:793–803
Heath DJ, Christian P, Griffin M (2002) Involvement of tissue trans glutaminase in the stabilisation of biomaterial/tissue interfaces important in medical devices. Biomaterials 23:1519–1526
Herman JF, Mangala LS, Mehta K (2006) Implications of increased tissue transglutaminase (TG2) expression in drug-resistant breast cancer (MCF-7) cells. Oncogene 25:3049–3058
Huang Y, Fan J, Yang J, Zhu GZ (2008) Characterization of GPR56 protein and its suppressed expression in human pancreatic cancer cells. Mol Cellular Biochem 308:133–139
Huang LH, Haylor JL, Hau Z, Jones RA, Vickers ME, Wagner B, Griffin M, Saint RE, Coutts IGC, El Nahas AM, Johnson TS (2009) Transglutaminase inhibition ameliorates experimental diabetic nephropathy. Kidney Int 76:383–394
Huang L, Haylor JL, Fisher M, Hau Z, El Nahas AM, Griffin M, Johnson TS (2010) Do changes in transglutaminase activity alter latent transforming growth factor beta activation in experimental diabetic nephropathy? Nephrol Dial Transplant 25:3897–3910
Janiak A, Zemskov EA, Belkin AM (2006) Cell surface transglutaminase promotes RhoA activation via integrin clustering and suppression of the Src-p190RhoGAP signaling pathway. Mol Biol Cell 17:1606–1619
Johnson KA, Terkeltaub R (2005) External GTP-bound transglutaminase 2 is a molecular switch for chondrocyte hypertrophic differentiation and calcification. J Biol Chem 280:15004–15012
Johnson TS, El-Koraie AF, Skill NJ, Baddour NM, El Nahas AM, Njloma M, Adam AG, Griffin M (2003) Tissue transglutaminase and the progression of human renal scarring. J Am Soc Nephrol 14:2052–2062
Jones RA, Kotsakis P, Johnson TS, Chau DY, Ali S, Melino G, Griffin M (2006) Matrix changes induced by transglutaminase 2 lead to inhibition of angiogenesis and tumor growth. Cell Death Differ 13:1442–1453
Kang SK, Yi KS, Kwon NS, Park KH, Kim UH, Baek KJ, Im MJ (2004) Alpha(1B)-adrenoceptor signaling and cell motility—GTPase function of G(h)/transglutaminase 2 inhibits cell migration through interaction with cytoplasmic tail of integrin alpha subunits. J Biol Chem 279:36593–36600
Kausar T, Sharma R, Hasan MR, Tripathi SC, Saraya A, Chattopadhyay TK, Gupta SD, Ralhan R (2011) Clinical significance of GPR56, transglutaminase 2, and NF-kappaB in esophageal squamous cell carcinoma. Cancer Invest 29:42–48
Kiraly R, Csosz E, Kurtan T, Antus S, Szigeti K, Simon-Vecsei Z, Korponay-Szabo IR, Keresztessy Z, Fesus L (2009) Functional significance of five noncanonical Ca-binding sites of human transglutaminase 2 characterized by site-directed mutagenesis. Febs J 276:7083–7096
Kleman JP, Aeschlimann D, Paulsson M, van der Rest M (1995) Transglutaminase-catalyzed cross-linking of fibrils of collagen V/XI in A204 rhabdomyosarcoma cells. Biochemistry 34:13768–13775
Kotsakis P, Wang Z, Collighan RJ, Griffin M (2010) The role of tissue transglutaminase (TG2) in regulating the tumour progression of the mouse colon carcinoma CT26. Amino Acids (In press)
Kumar A, Xu J, Brady S, Gao H, Yu D, Reuben J, Mehta K (2010) Tissue transglutaminase promotes drug resistance and invasion by inducing mesenchymal transition in mammary epithelial cells. PLoS One 5:e13390
Kuncio GS, Tsyganskaya M, Zhu J, Liu SL, Zern MA (1996) TNF-alpha modulates expression of the tissue transglutaminase gene in human HEPG2 cells. Hepatology 24: 813
Lai TS, Slaughter TF, Peoples KA, Hettasch JM, Greenberg CS (1998) Regulation of human tissue transglutaminase function by magnesium–nucleotide complexes. Identification of distinct binding sites for Mg-GTP and Mg-ATP. J Biol Chem 273:1776–1781
Lai TS, Hausladen A, Slaughter TF, Eu JP, Stamler JS, Greenberg CS (2001) Calcium regulates S-nitrosylation, denitrosylation, and activity of tissue transglutaminase. Biochemistry 40:4904–4910
Lorand L, Radek JT, Jeong JM, Murthy SNP, Ingham KC (1993) Affinity of human red-cell transglutaminase for a 42 k gelatin-binding fragment of human plasma fibronectin. Faseb J 7:A1280

Mangala LS, Mehta K (2005) Tissue transglutaminase (TG2) in cancer biology. Prog Exp Tumor Res 38:125–138

Mangala LS, Fok JY, Zorrilla-Calancha IR, Verma A, Mehta K (2007) Tissue transglutaminase expression promotes cell attachment, invasion and survival in breast cancer cells. Oncogene 26:2459–2470

Mehta K, McQueen T, Neamati N, Collins S, Andreeff M (1996) Activation of retinoid receptors RAR alpha and RXR alpha induces differentiation and apoptosis, respectively, in HL-60 cells. Cell Growth Differ 7:179–186

Mehta K, Fok JY, Mangala LS (2006) Tissue transglutaminase: from biological glue to cell survival cues. Front Biosci 11:173–185

Mehta K, Kumar A, Kim HI (2010) Transglutaminase 2: a multitasking protein in the complex circuitry of inflammation and cancer. Biochem Pharmacol 80:1921–1929

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

Mishra S, Murphy LJ (2006) The p53 oncoprotein is a substrate for tissue transglutaminase kinase activity. Biochem Biophys Res Comm 339:726–730

Mycek MJ, Clarke DD, Neidle A, Waelsch H (1959) Amine incorporation into insulin as catalyzed by transglutaminase. Arch Biochem Biophys 84:528–540

Nadalutti C, Viiri KM, Kaukinen K, Maki M, Lindfors K (2011) Extracellular transglutaminase 2 has a role in cell adhesion, whereas intracellular transglutaminase 2 is involved in regulation of endothelial cell proliferation and apoptosis. Cell Prolif 44:49–58

Nakano Y, Forsprecher J, Kaartinen MT (2010) Regulation of ATPase activity of transglutaminase 2 by MT1-MMP: implications for mineralization of MC3T3–E1 osteoblast cultures. J Cell Physiol 223:260–269

Nelea V, Nakano Y, Kaartinen MT (2008) Size distribution and molecular associations of plasma fibronectin and fibronectin crosslinked by transglutaminase 2. Protein J 27:223–233

Peng XJ, Zhang YH, Zhang HF, Graner S, Williams JF, Levitt ML, Lokshin A (1999) Interaction of tissue transglutaminase with nuclear transport protein importin-alpha 3. FEBS Lett 446:35–39

Quan G, Choi JY, Lee DS, Lee SC (2005) TGF-beta up-regulates transglutaminase two and fibronectin in dermal fibroblasts: a possible mechanism for the stabilization of tissue inflammation. Arch Dermatol Res 297:84–90

Santhanam L, Tuday EC, Webb AK, Dowzicky P, Kim JH, Oh YJ, Sikka G, Kuo M, Halushka MK, Macgregor AM et al (2010) Decreased *S*-nitrosylation of tissue transglutaminase contributes to age-related increases in vascular stiffness. Circ Res 107:117–125

Scarpellini A, Germack R, Lortat-Jacob H, Muramtsu T, Johnson TS, Billett E, Verderio EA (2009) Heparan sulphate proteoglycans are receptors for the cell-surface trafficking and biological activity of transglutaminase-2. J Biol Chem 284:18411–18423

Siegel M, Khosla C (2007) Transglutaminase 2 inhibitors and their therapeutic role in disease states. Pharmacol Ther 115:232–245

Smethurst PA, Griffin M (1996) Measurement of tissue transglutaminase activity in a permeabilized cell system: its regulation by Ca^{2+} and nucleotides. Biochem J 313:803–808

Sollid LM, Khosla C (2005) Future therapeutic options for celiac disease. Nat Clin Pract Gastroenterol Hepatol 2:140–147

Spurlin TA, Bhadriraju K, Chung KH, Tona A, Plant AL (2009) The treatment of collagen fibrils by tissue transglutaminase to promote vascular smooth muscle cell contractile signaling. Biomaterials 30:5486–5496

Stamnaes J, Pinkas DM, Fleckenstein B, Khosla C, Sollid LM (2010) Redox regulation of transglutaminase 2 activity. J Biol Chem 285:25402–25409

Stephens P, Grenard P, Aeschlimann P, Langley M, Blain E, Errington R, Kipling D, Thomas D, Aeschlimann D (2004) Crosslinking and G-protein functions of transglutaminase 2 contribute differentially to fibroblast wound healing responses. J Cell Sci 117:3389–3403

Suto N, Ikura K, Sasaki R (1993) Expression induced by interleukin-6 of tissue-type transglutaminase in human hepatoblastoma Hepg2 cells. J Biol Chem 268:7469–7473

Tanaka K, Yokosaki Y, Higashikawa F, Saito Y, Eboshida A, Ochi M (2007) The integrin alpha 5 beta 1 regulates chondrocyte hypertrophic differentiation induced by GTP-bound transglutaminase 2. Matrix Biol 26:409–418

Tatsukawa H, Fukaya Y, Frampton G, Martinez-Fuentes A, Suzuki K, Kuo TF, Nagatsuma K, Shimokado K, Okuno M, Wu J et al (2009) Role of ransglutaminase 2 in liver injury via cross-linking and silencing of transcription factor Sp1. Gastroenterology 136:1783–1795

Telci D, Wang Z, Li X, Verderio EA, Humphries MJ, Baccarini M, Basaga H, Griffin M (2008) Fibronectin-tissue transglutaminase matrix rescues RGD-impaired cell adhesion through syndecan-4 and beta1 integrin co-signaling. J Biol Chem 283:20937–20947

Telci D, Collighan RJ, Basaga H, Griffin M (2009) Increased TG2 expression can result in induction of transforming growth factor beta 1, causing increased synthesis and deposition of matrix proteins, which can be regulated by nitric oxide. J Biol Chem 284:29547–29558

Toth B, Sarang Z, Vereb G, Zhang AL, Tanaka S, Melino G, Fesus L, Szondy Z (2009) Over-expression of integrin beta 3 can partially overcome the defect of integrin beta 3 signaling in transglutaminase 2 null macrophages. Imm Lett 126:22–28

Van Herck JL, Schrijvers DM, De Meyer GR, Martinet W, Van Hove CE, Bult H, Vrints CJ, Herman AG (2010) Transglutaminase 2 deficiency decreases plaque fibrosis and increases plaque inflammation in apolipoprotein-e-deficient mice. J Vascular Res 47:231–240

Verderio E, Nicholas B, Gross S, Griffin M (1998) Regulated expression of tissue transglutaminase in Swiss 3T3 fibroblasts: effects on the processing of fibronectin, cell attachment, and cell death. Exp Cell Res 239:119–138

Verderio EA, Telci D, Okoye A, Melino G, Griffin M (2003) A novel RGD-independent cell adhesion pathway mediated by fibronectin-bound tissue transglutaminase rescues cells from anoikis. J Biol Chem 278:42604–42614

Verderio EA, Johnson T, Griffin M (2004) Tissue transglutaminase in normal and abnormal wound healing: review article. Amino Acids 26:387–404

Verma A, Wang H, Manavathi B, Fok JY, Mann AP, Kumar R, Mehta K (2006) Increased expression of tissue transglutaminase in pancreatic ductal adenocarcinoma and its implications in drug resistance and metastasis. Cancer Res 66:10525–10533

Verma A, Guha S, Diagaradjane P, Kunnumakkara AB, Sanguino AM, Lopez-Berestein G, Sood AK, Aggarwal BB, Krishnan S, Gelovani JG, Mehta K (2008) Therapeutic significance of elevated tissue transglutaminase expression in pancreatic cancer. Clinical Cancer Res 14:2476–2483

Wang Z, Collighan RJ, Gross SR, Danen EH, Orend G, Telci D, Griffin M (2010) RGD-independent cell adhesion via a tissue transglutaminase–fibronectin matrix promotes fibronectin fibril deposition and requires syndecan-4/2 and {alpha}5{beta}1 integrin co-signaling. J Biol Chem 285:40212–40229

Wang Z, Telci D, Griffin M (2011) Importance of syndecan-4 and syndecan-2 in osteoblast cell adhesion and survival mediated by a tissue transglutaminase–fibronectin complex. Exp Cell Res 317:367–381

Xu L, Begum S, Hearn JD, Hynes RO (2006) GPR56, an atypical G protein-coupled receptor, binds tissue transglutaminase, TG2,

and inhibits melanoma tumor growth and metastasis. Proc Natl Acad Sci USA 103:9023–9028

Yuan L, Choi KH, Khosla C, Zheng X, Higashikubo R, Chicoine MR, Rich KM (2005) Tissue transglutaminase 2 inhibition promotes cell death and chemosensitivity in glioblastomas. Mol Cancer Therapeut 4:1293–1302

Yuan L, Siegel M, Choi K, Khosla C, Miller CR, Jackson EN, Piwnica-Worms D, Rich KM (2007) Transglutaminase 2 inhibitor, KCC009, disrupts fibronectin assembly in the extracellular matrix and sensitizes orthotopic glioblastomas to chemotherapy. Oncogene 26:2563–2573

Zemskov EA, Loukinova E, Mikhailenko I, Coleman RA, Strickland DK, Belkin AM (2009) Regulation of platelet-derived growth factor receptor function by integrin-associated cell surface transglutaminase. J Biol Chem 284:16693–16703

Zemskov EA, Mikhailenko I, Hsia RC, Zaritskaya L, Belkin AM (2011) Unconventional secretion of tissue transglutaminase involves phospholipid-dependent delivery into recycling endosomes. PLoS One 6:e19414