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REVIEW ARTICLE

Transglutaminase 2 cross-linking of matrix proteins: biological significance and medical applications

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Abstract This review summarises the functions of the enzyme tissue transglutaminase (TG2) in the extracellular matrix (ECM) both as a matrix stabiliser through its protein cross-linking activity and as an important cell adhesion protein involved in cell survival. The contribution of extracellular TG2 to the pathology of important diseases such as cancer and fibrosis are discussed with a view to the potential importance of TG2 as a therapeutic target. The medical applications of TG2 are further expanded by detailing the use of transglutaminase cross-linking in the development of novel biocompatible biomaterials for use in soft and hard tissue repair.

Keywords Tissue transglutaminase · TG2 · Biomaterials · Extracellular matrix · Cross-linking

Introduction

The mammalian transglutaminases, to which tissue transglutaminase (TG2) belongs, are a family of enzymes each capable of covalently modifying proteins by cross-linking them via ε(γ-glutamyl)lysine bonds. These cross-links are resistant to proteolytic cleavage and also confer mechanical stability to aggregates of proteins which may contain many such cross-links. These properties are utilised in structures in the body such as skin, hair, and blood clots where they add an essential extra degree of strength and stability to existing macromolecular structures. There are currently eight described transglutaminase enzymes in mammals, six of which have been characterised to some degree (Griffin et al. 2002). Each enzyme has its own distinct target(s) for cross-linking and, consequently, function. All the mammalian transglutaminases require Ca^{2+} for activity, some also require proteolytic cleavage of propeptides, and three (TG2, TG3 and TG5) are inhibited by GTP. This review is focused on TG2, in particular its secretion from cells, its widely reported ability to adhere to and cross-link matrix proteins important in such physiological processes as matrix deposition and turnover (Zemskov et al. 2006) and in associated pathologies (Gentile and Cooper 2004; Facchiano et al. 2006; Johnson et al. 2007; Shweke et al. 2008; Stenberg et al. 2008). Importantly, it will also look how some of these properties have been harnessed in potential medical applications.

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TG2: a multifunctional enzyme

Tissue transglutaminase (TG2) is the ubiquitous member of this protein cross-linking family of enzymes and is also one of the most studied. Its role has yet to be fully elucidated although there is an abundance of literature describing its effects. In addition to its ability to cross-link proteins (Griffin and Wilson 1984; Griffin et al. 2002), it can also bind and hydrolyse GTP and ATP (Lee et al. 1993). In addition, it also has a protein disulfide isomerase activity (Hasegawa et al. 2003) and may even function as a protein kinase (Mishra and Murphy 2004). The GTPase activity has been linked to the function of TG2 as a G protein (Gαh) involved in signalling from $\alpha_{1B/D}$ adrenergic receptors to downstream effectors such as phospholipase Cδ1. TG2 has a high affinity fibronectin binding site located in the N-terminal domain, with peptide ^{88}WTATVVDQQDCTLSLQLTT^{106} being directly involved in this interaction (Hang et al. 2005).

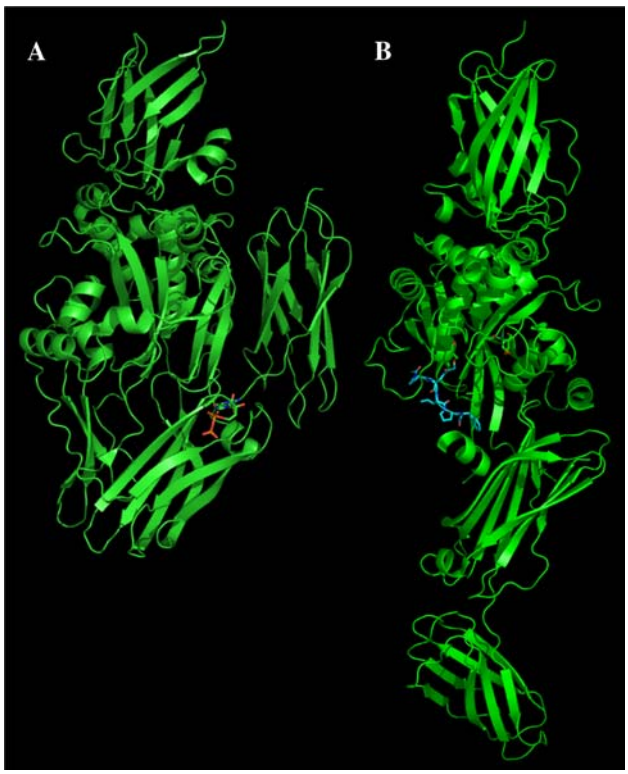

Fig. 1 Structures of tissue transglutaminase derived by X-ray crystallography (Liu et al. 2002; Pinkas et al. 2007). **a** GDP-bound bacterially expressed recombinant human TG2, **b** irreversible peptide inhibitor-bound bacterially expressed recombinant human TG2. The four domain structure of TG2 is clearly seen in both structures, although trapping of the irreversible gluten peptide mimetic inhibitor Ac–P(DON)LPF-NH_2 in the active site of TG2 results in the stable extended conformation seen in **(b)**, compared to the 'inactive' compact GDP-bound conformation observed in **(a)**. The extended conformation is possibly stabilised by a vicinal disulphide bond between Cys370 and Cys371 and it seems likely that this is also the reason for the requirement of reducing agents to restore the activity of purified TG2

Structure of TG2

The structure of TG2 is highly conserved between the transglutaminases, with four distinct domains (Fig. 1). TG2 has been crystallised in two different conformations which probably represent the two extremes of movement. The compact GTP bound conformation represents the intracellular form of TG2 where GTP is prevalent, whereas the extended oxidised form may represent a mature extracellular form, depending on the redox potential of the local environment. It is important to consider that these two conformations may be important in its varied cellular functions since they generate different interactions with other components of the cell surface and ECM, with juxtaposition of multiple binding partners also being altered.

Secretion of TG2

Initially reported to be an intracellular enzyme with intracellular functions, it has now been widely accepted that TG2 has an extracellular role in keeping with its regulation by Ca^{2+} and GTP. TG2 has no signal sequence to direct it to the ER/Golgi and it has never been observed in these structures. TG2 and Factor XIII A subunit are secreted via an uncharacterized non-classical secretory pathway (Gaudry et al. 1999a, b; Akimov and Belkin 2001a, b). TG2 and FXIIIA lack an ER targeting signal sequence and there is no evidence of their glycosylation (Folk and Finlayson 1977; Ichinose et al. 1990). Little information exists as to the secretory mechanism of TG2 and FXIIIA. FXIIIA can be expressed and secreted independently of FXIIIB (Al-Jallad et al. 2006), the non-catalytic subunit of plasma FXIII, which is secreted via the normal ER/Golgi classical secretory pathway (Kaetsu et al. 1996). It is found on the surface of monocytes (Akimov and Belkin 2001a, b) and is actively secreted from differentiating osteoblasts, together with TG2, in response to stimulus by ascorbic acid (Al-Jallad et al. 2006). In addition to its role in the stabilization of fibrin in blood clots, extracellular FXIIIA is also involved in cellular adhesion (Ueki et al. 1996; Akimov and Belkin 2001a, b), wound healing and angiogenesis (Jones et al. 2006; Dardik et al. 2006; Dardik et al. 2007), osteoblast differentiation and matrix mineralization (Al-Jallad et al. 2006). TG2 is normally localised within cells in three major cell compartments (cytosol, plasma membrane/extracellular matrix, and nucleus). In addition, despite having glycosylation sites, it is not glycosylated (Folk and Finlayson 1977). It has been hypothesised that TG2 is secreted from cells via a non-classical pathway, although no evidence has been published in support of any mechanism.

With respect to TG2 secretion, early data indicate that membrane translocation and deposition into the ECM requires an intact N-terminal β-sandwich domain and the active site conformation of the enzyme (Gaudry et al. 1999a, b; Balklava et al. 2002), whilst other data suggest TG secretion is in some way associated with Integrin $\beta 1$ (Akimov et al. 2000). The presence of a non-proline cis peptide bond (Y274) is required not just for activity, but also for secretion (Balklava et al. 2002; Johnson and Terkeltaub 2005). This bond, which is also found in FXIIIA, is thought to be important in the transition between the different conformations (Weiss et al. 1998). Very recent evidence suggests that TG2 is externalised as a result of cell damage as part of a membrane repair process (Kawai et al. 2008) and is possibly involved in the modulation of autoimmunity by preventing the release of cytosolic contents which has been commented on previously (Nicholas et al. 2003). Externalised transglutaminase does not leak out of the cell since mutations have been made that do not

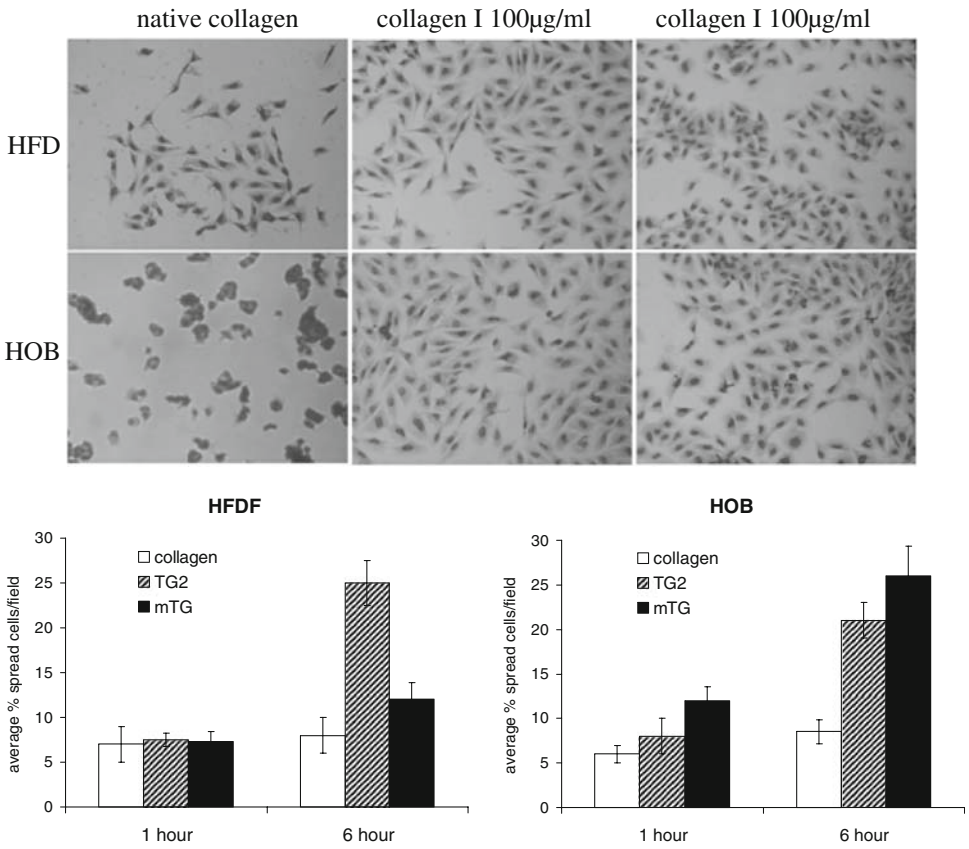

Fig. 2 Spreading of human osteoblasts (HOB) and human foreskin dermal fibroblasts (HFDF) cultured on native collagen I and, collagen I cross-linked with either 100 µg/ml TG2 or 100 µg/ml mTG (cell seeding density of 2×10^5 cells/cm^2). The *upper panel* shows representative photographs (×400) of fixed cells after 6 h incubation. The *graphs* show the average percentage of cells per field of view that were spread at 1–6 h time points. Modified from Chau et al. (2005)

affect its intracellular distribution, but prevent it from being externalised (Balklava et al. 2002; Johnson and Terkeltaub 2005). In neuroblastoma cells treated with the neurotoxin 1-methyl-4-phenylpyridinium (MPP) TG2 has been shown to be activated and redistributed to ER granules (Wilhelmus et al. 2007). Possible regulation of the turnover of extracellular TG2 may be via endocytosis, promoted by binding to the low-density lipoprotein receptor-related protein 1 (LRP1) and subsequent degradation in lysosomes (Zemskov et al. 2007).

TG2 in the ECM

Cell surface TG2 is involved in cell adhesion via its tight interaction with fibronectin (Gaudry et al. 1999a, b) and the resultant intracellular signalling effects are reported to be mediated by various $\beta1$ and $\beta3$ integrins (Gaudry et al. 1999a, b; Isobe et al. 1999; Akimov et al. 2000; Takahashi et al. 2000; Tanaka et al. 2007). The interaction of cell surface TG2 with fibronectin promotes more rapid assembly of fibronectin mediated by $\alpha5\beta1$ integrin, although TG2 cannot substitute for this integrin (Akimov and Belkin 2001a, b). The transamidating activity of TG2 is not required (Isobe et al. 1999; Akimov and Belkin 2001a, b) and TG2 is thought to remain associated with the assembled fibronectin fibres (Akimov and Belkin 2001a, b). Since the assembly of fibronectin is the initiator for a number of other ECM structures such as fibrillin 1, TG2 has the potential to play a major role in initial assembly of the ECM, not just in modification of the existing ECM. TG2, once deposited into the matrix and in complex with fibronectin, can also bind to the heparan sulphate chains of syndecan 4 on the cell surface (Verderio et al. 2003). This process is independent of RGD-mediated fibronectin binding and is responsible for the observation that added TG2 can restore cell adhesion to fibronectin in the presence of inhibitory RGD peptides. Binding by this mechanism results in protein kinase C alpha (PKCα) activation and its subsequent interaction with $\beta1$ integrin, which reinforces actin stress fibre organisation. Cell signalling via this pathway leads to FAK and ERK1/2 kinase activation. It is proposed that TG2 released under conditions of wounding or matrix turnover can both enhance and/or substitute for the normal mode of integrin associated cell adhesion and associated intracellular signalling, promoting cell survival (Telci et al. 2008).

Wound healing

The involvement of TG2 in the wound healing process has been described in detail elsewhere (Verderio et al. 2005;

Telci and Griffin 2006) and will only be briefly covered here. It has been demonstrated that TG2 is secreted into the ECM after either chemical or mechanical injury of fibroblasts (Upchurch et al. 1987, 1991), where it becomes associated with fibronectin. In a rat model of dermal wound healing, it was shown that TG2 activity is increased in all layers of the skin and that most activity was associated with insoluble residue remaining after detergent extraction, suggestive of cross-linked proteins (Bowness et al. 1988). In skin regenerating from cultured epithelial autografts in human burn patients, transglutaminase activity attributed to TG2 was localised to the microfibrillar apparatus of the papillary dermis and surrounding capillaries. The appearance of TG2 at the dermo-epidermal junction and its associated cross-linking were also correlated with stabilisation of keratinocyte autografts (Raghunath et al. 1996). It has been postulated that TG2 cross-linking of lethally damaged cells to produce stable structures with little release of cell contents is involved in maintaining tissue integrity and reducing immune responses after necrosis following loss of cellular Ca^{2+} homeostasis (Nicholas et al. 2003; Fesus and Szondy 2005).

Involvement of TG2 in matrix associated pathologies

Cancer

The ability of malignant cells to proliferate and invade surrounding tissues is characterised by their insensitivity to growth signals and by their resistance to apoptotic cell death (Hanahan and Weinberg 2000), with the GTP binding and transamidating properties of TG2 having a potential role in these functions (Birckbichler and Patterson 1978). Overexpression of TG2 and a cross-linking defective mutant (C277S) in malignant hamster fibrosarcoma cells (MetB) leads to delayed progression from S-phase to G_2/M (Mian et al. 1995), which is in keeping with the observation that reduced TG2 activity is associated with tumour growth and metastasis (Barnes et al. 1985; Knight et al. 1991). Intracellular TG2 has, however, been suggested as both a pro- and anti-apoptotic factor by several reports (Mehta 1994; Piacentini et al. 1996; Oliverio et al. 1997; Nicholas et al. 2003), although increased TG2 expression has been correlated with increased apoptotic index in human breast carcinomas (Grigoriev et al. 2001). Cytosolic TG2 can be anti-apoptotic via activation of the NF kappa B pathway (Mann et al. 2006; Condello et al. 2008). In its extracellular form, TG2/fibronectin is also associated with a cell survival role via its interaction with integrins (Kotsakis and Griffin 2007). The RGD-independent binding of TG2/fibronectin via syndecan 4 could also lead to cell survival signalling (Telci et al. 2008). High expression of TG2 by breast cancer cells is reported to increase their survival, invasion and motility. In these cells TG2 was observed on the surface in close association with β1, β4 and β5 integrins. Downregulation of endogenous TG2 by siRNA inhibited fibronectin mediated cell attachment, survival and invasion, whereas overexpression of TG2 improved invasion and attachment to fibronectin (Herman et al. 2006; Mangala et al. 2007).

It has been proposed that digestion of cell surface TG2 by MT-MMPs is involved in the regulation of cancer cell attachment and locomotion. Digestion of cell surface TG2 by MT1-MMP suppressed cell adhesion and locomotion on fibronectin, whereas fibronectin protected cell surface TG2 from MT1-MMP digestion, promoting cell adhesion and locomotion (Belkin et al. 2001). The effect of exogenously adding TG2 in two models of angiogenesis, a process essential to tumour progression, demonstrated that endothelial tube formation was inhibited and that there was an accumulation of cross-linked ECM proteins without causing an increase in cell death (Jones et al. 2006). Similarly, injection of TG2 into mice with CT26 colon carcinoma tumours caused a reduction of organised vasculature, an increase in fibrotic-like tissue, an increase in $\varepsilon(\gamma$-glutamyl)lysine cross-links, a reduction in tumour growth and in some cases regression, whereas the same treatment applied to TG2 knockout mice showed increased tumour growth and increased mortality (Jones et al. 2006). In this study the authors also allude to a mechanism whereby the increase in matrix rigidity caused by TG2 cross-linking is responsible for the alteration in cell behaviour observed, since only the activity of TG2 was required. In another study of 200 cases of human breast cancer, an increased TG2 level in the stroma surrounding the tumour was associated with a reduction in lymph node metastasis. A breast cancer cell line (MDA-MB-231) also showed strong inhibition of invasion in an in vitro Matrigel Transwell invasion assay when the matrix was cross-linked with TG2 (Mangala et al. 2005).

Fibrosis and scarring

TG2 is involved in many pathological conditions where it is thought that the wound-healing response does not progress normally. Chronic stress resulting in fibrotic disease is caused by an excess of ECM which is cross-linked by TG2 (Griffin et al. 1979; Richards et al. 1991; Johnson et al. 1997, 1999; Mirza et al. 1997; Grenard et al. 2001; Skill et al. 2001). TG2 has been shown to have a role in the storage and activation of TGFβ1 in the ECM, where the large latent TGFβ1 binding protein-1 (LTBP-1) is cross-linked to matrix proteins. It was shown that LTBP-1 colocalised with TG2 on the basal and apical surfaces of cells as well as at cell-cell contacts and that LTBP-1 also colocalised with intra- and extracellular fibronectin,

including large cross-linked complexes of cells over-expressing TG2 (Verderio et al. 1999). TG2 knockout mice showed reduction in active TGFβ1 in a model of renal fibrosis induced by ureteral obstruction. This was accompanied by reduced infiltration of macrophages and myofibroblasts, a reduction in total collagen deposition and collagen I biosynthesis (Shweke et al. 2008). In addition to causing increased matrix deposition through activation of TGFβ1, TG2 cross-linking of the ECM has been shown to result in a reduction in matrix turnover leading to net deposition and accumulation, the increased stability of the cross-linked ECM being responsible (Gross et al. 2003; Jones et al. 2006).

TG2 has also been implicated in the progression and stabilisation of atherosclerotic plaques, where cross-linking is thought to have a role in preventing plaque rupture (Haroon et al. 2001). Elevated TG2 and fibronectin levels accompanied by $\varepsilon(\gamma$-glutamyl)lysine cross-links are found in glaucomatous human trabecular meshwork cells and tissues, implicating TG2 in glaucoma (Welge-Lussen et al. 2000; Tovar-Vidales et al. 2008). Hypertrophic scarring, which is characterised by excessive collagen deposition by the prolonged action of myofibroblasts, also involves TG2 (Linge et al. 2005), a process which can be prevented by the application of transglutaminase inhibitors (Dolynchuk et al. 1996). The importance of TG2 activity in the progression of renal fibrosis has been highlighted by the beneficial effects of TG inhibition in this condition. The use of an existing membrane soluble irreversible TG inhibitor, R283 or 1,3-dimethyl-2[(oxopropyl)thio]-imidazolium (Freund et al. 1994) and a novel membrane-impermeable irreversible TG inhibitor R281, or N-benzyloxycarbonyl-L-phenylalanyl-6-dimethylsulphonium-5-oxo-L-norleucine (Griffin et al. 2008) both caused a reduction in TG activity, ECM cross-link, and reduced glomerular and interstitial scarring in a rat subtotal nephrectomy model of chronic renal fibrosis (Fig. 3). Targeting of the extracellular TG with the membrane-impermeable inhibitor did not affect the transcription of major ECM proteins or MMP-1, nor in this model did it affect the activity of TGFβ1, suggesting that in this model it was mainly the cross-linking activity of TG2 on the ECM that was responsible for the effect (Johnson et al. 2007). The use of TG2 inhibitors therefore shows great promise for the treatment of renal fibrosis and indeed other fibrotic diseases characterised by increased ECM deposition and cross-linking, a fact further highlighted by the finding that TG2 knockout mice show a reduction of renal fibrosis induced by ureteral obstruction (Shweke et al. 2008). The major component of fibrotic tissue is fibrillar collagen, in particular collagen I. It is therefore important to understand the effects of TG2 cross-linking on both the physical and biological properties of collagen. This will be discussed in the next section since collagen is one of the major components of artificial cell scaffolds for tissue engineering.

Medical applications: TG2 as a cross-linker of biomaterials

Given the importance of TG2 as a prolific cross-linker of matrix proteins it is not surprising that its use in the generation of naturally cross-linked biomaterials has been widely investigated. Most polymers used in tissue repair suffer from inadequate mechanical properties and biopolymers are often easily degraded. To prevent this problem, many of these materials are cross-linked using chemical or physical methods which can leave toxic residues and/or precipitate immune responses in the host (Chau et al. 2005). Transglutaminase has the ability to cross-link biopolymers in order to increase strength and resistance to proteolytic digestion (Chau et al. 2005; Jones et al. 2006). Since TG2 and $\varepsilon(\gamma$-glutamyl)lysine cross-linked proteins are found naturally within the body and are important in the wound-healing response, it is not unreasonable to assume that transglutaminase cross-linked biomaterials would have improved biocompatibility. A secondary, but very important effect of the presence of TG2 in a biomaterial, is that it can not only continue to cross-link matrix protein that it encounters, but the adhesion properties of TG2 when in complex with fibronectin can enhance the attachment, spreading and proliferation of inwardly migrating cells.

A TG2/fibronectin heteropolymer can be used to coat poly(ε-caprolactone) or other devices in order to improve biocompatibility and stabilise the implant/tissue interface. For example, human osteoblasts show increased cell spreading compared to either untreated or FN coated PCL (Heath et al. 2002). This is probably due to the TG2/FN complex binding to cell surface heparan sulphate proteoglycans such as syndecan 4, which osteoblasts express (Telci et al. 2008; Verderio et al. 2008). Overexpression of TG2 in a variety of cell lines grown on the polymers poly(DL lactide co-glycolide), poly(ε-caprolactone) and poly(L lactide) showed that human endothelial like cells (ECV-304) showed greatly improved attachment and spreading on all polymers and glass with increased TG2 expression, an effect shared by Swiss 3T3 fibroblasts on glass and PLA, and human osteoblasts on glass and PLG (Verderio et al. 2001). This study highlighted the importance of the distinct adhesive properties of TG2 that are independent of its cross-linking function, first observed with the increased cell attachment to plastic coated with either native or catalytically inactive TG2 (Isobe et al. 1999). Other attempts to functionalise biomaterials via TG2 cross-linking include the cross-linking of TG substrate peptides and peptide functionalised PEG onto cartilage to

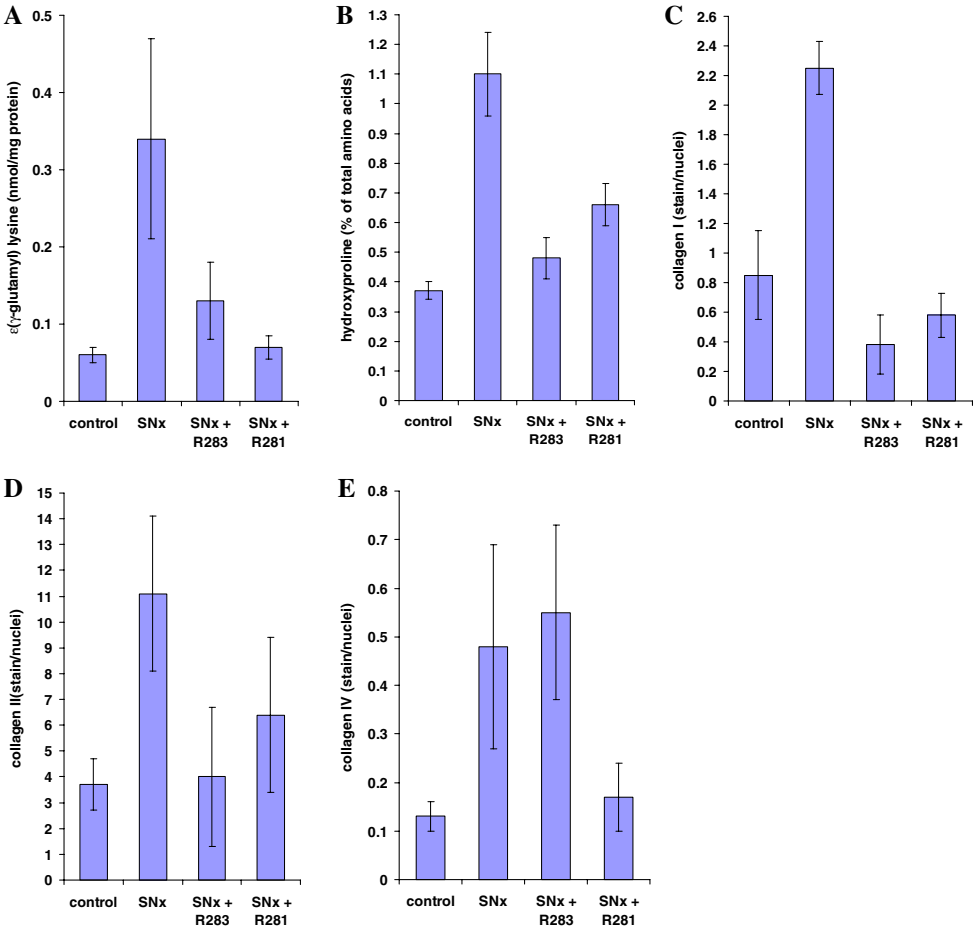

Fig. 3 Inhibition of TG2 by irreversible inhibitors R281 and R283 in a rat subtotal nephrectomy model of chronic renal fibrosis after 84 days. **a** ε(γ-glutamyl)lysine cross-links in kidney measured by HPLC, **b** total collagen measured by hydroxproline content, **c–e** collagen I, II and IV content, respectively, measured by immunohistochemistry. Control animals were subjected to a sham operation, SNx animals had left kidneys and 5/6 of the remaining kidney removed, inhibitor treated animals had either R281 or R283 delivered directly to the remaining kidney via osmotic minipumps. Modified from Johnson et al. (2007)

demonstrate that both lysine and glutamine substrates were reactive and could be incorporated to an average depth of 10 μm below the surface. The cartilage components fibronectin, collagen II, osteonectin, and osteopontin were all identified as being reactive towards the peptides in solution, showing that TG2 could theoretically cross-link to the majority of cartilage components (Jones and Messersmith 2007). TG2 has also been used as biological glue in the repair of articular cartilage. Cartilage is subject to high stresses in vivo and it is no surprise that TG2 is present in the ECM of articular cartilage and that it is the main transglutaminase activity found there (Summey et al. 2002). When TG2 was used to glue two pieces of cartilage together, the adhesive strength increased with TG2 concentration by as much as 40% when the cartilage was pretreated with chondroitinase AC or hyaluronidase to remove glycosaminoglycan chains of proteoglycans and was greater than that achieved with a commercial tissue sealant (Jurgensen et al. 1997). The use of TG2 cross-linked synthetic elastin-like polypeptide (ELP) as an injectable cartilage repair material is discussed below.

TG2 is particularly attractive for the formation of polymer hydrogels that solidify from a liquid form after introduction into the body via syringe or needle. Several different synthetic and bio-polymers have been investigated and these are discussed below.

Polymer hydrogels

Poly(ethyleneglycol)

Poly(ethyleneglycol) (PEG) is a simple, water-soluble, linear polymer which exhibits good biocompatibility and hydrophilicity. A major advantage is that it can easily be chemically modified to form water-insoluble hydrogels. For these reasons PEG has been widely used in biomaterials, particularly for drug delivery. An ideal polymer for functionalising with TG2 substrate groups, PEG was end-functionalised with glutaminimide to provide glutamine sites. The resulting PEG-Q was then cross-linked with a lysine-containing polypeptide by TG2. Whilst TG2 could catalyse the formation of clear hydrogels, the gelation rate was too slow to be useful (Sperinde and Griffith 1997). A subsequent attempt utilised two separately functionalised PEG-peptide conjugates, one linear PEG with two

H_2N-(L-DOPA)-FKG-NH_2 peptides per terminus and a four-armed PEG with single H_2N-GLQQQGAc peptides per terminus. When mixed together in equimolar quantities, a hydrogel was formed within minutes under physiological conditions, demonstrating that useful injectable hydrogels can be formed using TG2 (Hu and Messersmith 2003). In order to test the ability of this hydrogel to adhere to biological surfaces, the same authors mixed the two PEG-peptide conjugates with TG2 and used the solution to glue either guinea pig skin or bovine type I collagen film. The shear stress of the hydrogel-glued guinea pig skin was similar to that glued with a commercial fibrin sealant or TG2 alone, whereas the shear stress of hydrogel-glued type I collagen film was much greater than that observed with fibrin glue (Hu and Messersmith 2005).

Collagen/gelatin

Collagen has great potential as a biomaterial due to its mode of interaction within the body, exhibiting biodegradability, weak antigenicity and superior biocompatibility compared with other natural polymers. Moreover, the primary and probably most important reason for the usefulness of collagen in biomedical applications is that collagen can form fibres with extra strength and stability through its self-aggregation and cross-linking. Extracted collagen has poor mechanical properties, poor thermostability and is easily broken down by proteases. Cross-linking of collagen improves the stability of extracted collagen, preventing its rapid absorption and breakdown in the body. However, residual chemicals are often toxic or cause inflammatory responses to the host if not fully removed (Chau et al. 2007).

Early studies on the ability of TG2 to cross-link collagen suggested that native collagen and its fibrils were not a substrate for either TG2 or FXIIIA, although TG2 was able to cross-link denatured collagen effectively (Jelenska et al. 1980). Subsequent work showed that TG2 was able to incorporate putrescine into the aminopeptide of collagen III (Bowness et al. 1987) and cross-link the α1 chains of native collagen V and XI, although the glutamine acceptor sites were again shown to be in the non-helical propeptide domains (Kleman et al. 1995). More recently, direct evidence for the cross-linking of type I collagen was obtained (Collighan et al. 2004; Orban et al. 2004; Chau et al. 2005). It was shown that the addition of transglutaminase to neutralised acid soluble collagen I allowed cross-linking of individual tropocollagen monomers during assembly of the fibrils. The maximum cross-link incorporation was therefore greatly increased compared to treatment of native fibrils and was in the order of 1 cross-link per collagen (Collighan et al. 2004). The rate of collagen fibrillogenesis was also increased by the cross-linking activity of TG2 in a manner that was sensitive to specific TG2 inhibitors. TG2-treated ECM produced by dermal fibroblasts was shown to be significantly more resistant to MMP-1 digestion (Jones et al. 2006). The burst strength of sealed collagen I tubes was increased from 46 $\pm$ 3 to 71 $\pm$ 3 mmHg by the action of TG2 during fibrillogenesis and the denaturation temperature measured by differential scanning calorimetry increased from 37°C for native collagen to 65°C for a 50:1 collagen:TG2 mixture (Orban et al. 2004). It is clear that not only does collagen have the potential to be cross-linked with TG2 during ECM maturation and can potentially alter its physical properties, but it can also alter its biocompatibility. Both TG2 and $\varepsilon(\gamma$-glutamyl)lysine cross-linked proteins are found naturally within the body and are important in the wound-healing response, so it is therefore not unreasonable to assume that transglutaminase cross-linked biomaterials would have improved biocompatibility. Indeed when either human dermal fibroblasts or osteoblasts are plated onto collagen cross-linked by TG2, they show increased adhesion and spreading and enhanced proliferation (Fig. 2) (Chau et al. 2005). Importantly, from the point of view of bone healing and repair, where collagen sponges are frequently used (Friess 1998), osteoblasts demonstrate an increased propensity to differentiate when plated on TG2 cross-linked collagen compared to collagen alone (Chau et al. 2005). Whilst this effect can be explained by the masking/exposure of cell interaction sites, it may also be explained by the observation that cells can sense the elasticity of the matrix and this can not only modulate phenotypic changes, but can even determine stem cell lineage specification (Engler et al. 2006). Since TG2 cross-linking of matrices significantly alters their elasticity, this alone may be sufficient for many of the effects observed.

Gelatin, which is also a good substrate for transglutaminase, differs from collagen in that it has been partially hydrolysed to aid its solubility, although it retains many of the chemical characteristics of collagen. Since gelatin is easier to handle, it has been widely explored as a biomaterial, although it must be cross-linked to reduce its solubility in vivo. Gelatin is a good substrate for transglutaminases and this has been capitalised on by several studies, mainly with *Streptomyces mobaraensis* transglutaminase (mTG) or FXIIIA. Studies demonstrating the effects of TG2 cross-linking of gelatin are rare, although it has been shown that heat denatured collagen is a good substrate for TG2 (Jelenska et al. 1980). A recent study comparing the effects of TG2 and mTG on the cross-linking of purified porcine skin gelatin found that both enzymes had a similar reactivity towards the substrate, although further analysis of the cross-linked material was limited to mTG (Bertoni et al. 2006). The encouraging data obtained from biocompatibility studies of gelatin

cross-linked with mTG (Zhu and Tramper 2008) are therefore likely to translate to TG2 cross-linked gelatin.

Collagen-mimetic dendrimers

The use of dendrimers to replace collagen, but still retain its biocompatible properties, is the subject of much recent research since their use was proposed in 2002 (Kinberger et al. 2002; Goodman et al. 2003). A collagen dendrimer based on a poly(amidoamine) core (PAMAM) with pendant poly(Gly-Pro-Nleu) peptides has been described. The PAMAM core is thought to aid organisation of the peptides into a closely packed array with α-helical character similar to collagen and also allows interaction between helices to mimic native collagen fibrillar architecture (Kinberger et al. 2006). In order to increase the melting temperature of the collagen dendrimers from around 28°C as in the initial study, TG2 was used to cross-link engineered substrate sequences. PAMAM dendrimers were conjugated separately with the two peptides $(GPO)_3GFOGER(GPO)_3$ APQQEA and $(GPO)_3GFOGER(GPO)_3EDGFFKI$, containing the collagen $\alpha_1\beta_1$, $\alpha_1\beta_1$ and $\alpha_{11}\beta_1$ integrin-binding sequence GFOGER and TG substrate sequences APQQEA from osteonectin and EDGFFKI from fibrillin-1. The two dendrimers were cross-linked together in equimolar amounts to produce a product with stable α-helical conformation and a melting temperature of 38°C. Cell binding assays with human hepatocarcinoma cells (Hep3B) demonstrated that cell adhesion to cross-linked dendrimers was approximately 80% of that to native type I collagen and was greater than either of the two non-cross-linked dendrimers, suggesting that TG2 cross-linking enhances cell adhesion, possibly by integrin clustering or optimisation of the GFOGER triple helical conformation (Khew et al. 2008). These materials could show significant promise as alternatives to collagen.

Self-assembling peptides

Self-assembling peptides are those that can organise themselves into complex nanostructures with either α-helical or, more commonly, β-sheet character, such as fibrillar networks or hydrogels (Holmes 2002). They have been used as three dimensional matrices and support growth and differentiation of neuronal cells (Holmes et al. 2000) and chondrocytes (Kisiday et al. 2002). Peptides are designed so that hydrophilic side chains and hydrophobic side chains are positioned on opposite sides of the β-sheet, allowing self-assembly of a wide variety of sequences. The peptide Ac-QQKFQFQFEQQ-Am was shown to self-assemble into a tangled network of 20 nm diameter fibrils with β-sheet secondary structure. Reaction of the monomeric and assembled peptide with monodansylcadaverine (MDC) and TG2 demonstrated that five glutamines were reactive. The peptide n-dansyl-GLKGGRGDS-Am, containing a lysine donor TG2 substrate site and an integrin-binding RGD motif separated by a diglycinyl spacer, was also able to be cross-linked by TG2 to five glutamines of the self-assembling peptide (Collier and Messersmith 2003).

Elastin-like polypeptides (ELP)

Elastin-like polypeptides are composed of oligomeric repeats of the sequence Val-Pro-Gly-Xaa-Gly, where Xaa is any amino acid except proline, which is found in elastin, a component of muscle, ligaments, cartilage and many other soft tissues. At low temperatures, the ELP solution has low viscosity and can easily be mixed with cells prior to injection, where the higher temperature causes spontaneous aggregation of peptides to form a gel-like coacervate. The ELP sequence can be modified to control the mechanical properties of the resulting gel, although chemical cross-linking is required to achieve a satisfactory material for cartilage repair. Two ELP peptides with sequences $[VPGKG(VPGVG)_6]_{16}$ and $[VPGQG(VPGVG)_6]_{16}$ were designed with lysine and glutamine substrates, respectively. TG2 cross-linking of a 1:1 mixture of these peptides with isolated porcine intervertebral disc fibrochondrocytes resulted in an insoluble gel with improved mechanical characteristics that retained the ability to sustain the fibrochondrocytic phenotype (McHale et al. 2005). It is likely that some optimisation of the TG2 substrate sequences would result in a more cross-linked ELP gel, since it was also noted that the mechanical properties of the TG2 cross-linked gel were less satisfactory than a chemically cross-linked gel.

Microbial TG

The initial emergence of *Streptomyces mobaraensis* transglutaminase (mTG) related to its use as a food protein cross-linking agent, capitalising on its cheap and plentiful production, its small size, its independence from cofactors and its broad substrate specificity (Collighan et al. 2002). It has seen recent industrial use in other areas such as textiles and biomaterials for the same reasons. Although it is likely that mTG and TG2 show different reactivities towards glutamine and lysine residues in the same substrate, mTG cross-linking may be expected to be equivalent to TG2 cross-linking, but without the added effects of residual TG2 as a cell binding protein. For this reason mTG cross-linked biomaterials can produce valuable observations in this respect. Since it is impossible to discuss TG2 applications without some appreciation of the considerable work

already performed with mTG, we will briefly describe some relevant examples using mTG, although see (Zhu and Tramper 2008) for a broader overview of the applications of mTG.

Gelatin is a good substrate for mTG (Fuchsbauer et al. 1996) and it has been observed that gelatin cross-linked with mTG demonstrated a significant increase in storage modulus and thermal stability, comparable to that obtained with glutaraldehyde cross-linking. In addition, mTG cross-linking did not affect the cytocompatibility of matrices (Broderick et al. 2005), with improvements in cell adhesion possible by incorporation of RGD peptides (Ito et al. 2003) or laminin peptides (Damodaran et al. 2008). Collagen I cross-linked with mTG shows similar properties to that treated with TG2 (Chau et al. 2005; Garcia et al. 2007) and such biomaterials have been tested in vivo in a rat model of dermal wound-healing, where it was found that they resisted enzymatic degradation and stimulated wound bed angiogenesis and epithelialisation, without any significant inflammatory reactions. In addition, there was a reduction in collagen III deposition and wound contraction, indicative of a beneficial moderation of the fibroproliferative phenotype (Garcia et al. 2008). Collagen II has also been investigated as a substrate for mTG, with similar success (O'Halloran et al. 2006). Mixtures of collagen II, aggrecan and hyaluronan cross-linked with mTG were suitable for the support of bovine nucleus pulposus cells in the development of an injectable intervertebral disc repair (O'Halloran et al. 2008). The use of mTG in the generation of composite biomaterials is exemplified by the generation of melt-extruded nerve guide tubes for peripheral nerve repair using blends of poly(ε-caprolactone) and gelatin cross-linked with mTG. The gelatin domains were further functionalised with a poly L-lysine peptide to greatly enhance the adhesion and proliferation of neuroblastoma cells (Chiono et al. 2008).

It is clear that mTG has demonstrated great potential as a biomaterials cross-linker and does not demonstrate cytotoxicity to a variety of cell types in vitro at effective cross-linking concentrations. However, from an ethical point of view, it remains likely that future in vivo applications would require the use of a mammalian transglutaminase.

Conclusion

This review has demonstrated the functional importance of TG2 as an extracellular protein both in the cross-linking and stabilisation of matrix proteins during normal physiological processes but also as an important matrix associated cell adhesion protein involved in cell survival. It has also alluded to the importance of TG2 in pathological situations such as fibrosis and cancer and highlighted the value of TG2 as a therapeutic target in the treatment of these diseases. Importantly, by harnessing the cross-linking activity of TG2 it has further demonstrated the medical applications of TG2 in the modification of a wide range of natural and synthetic biomaterials.

References

Akimov SS, Belkin AM (2001a) Cell-surface transglutaminase promotes fibronectin assembly via interaction with the gelatin-binding domain of fibronectin: a role in TGF beta-dependent matrix deposition. J Cell Sci 114(16):2989–3000

Akimov SS, Belkin AM (2001b) Cell surface tissue transglutaminase is involved in adhesion and migration of monocytic cells on fibronectin. Blood 98(5):1567–1576

Akimov SS, Krylov D, Fleischman LF, Belkin AM (2000) Tissue transglutaminase is an integrin-binding adhesion co-receptor for fibronectin. J Cell Biol 148(4):825–838

Al-Jallad HF, Nakano Y, Chen JL, McMillan E, Lefebvre C, Kaartinen MT (2006) Transglutaminase activity regulates osteoblast differentiation and matrix mineralization in MC3T3–E1 osteoblast cultures. Matrix Biol 25(3):135–148

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(19):16567–16575

Barnes RN, Bungay PJ, Elliott BM, Walton PL, Griffin M (1985) Alterations in the distribution and activity of transglutaminase during tumor-growth and metastasis. Carcinogenesis 6(3):459–463

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(21):18415–18422

Bertoni F, Barbani N, Giusti P, Ciardelli G (2006) Transglutaminase reactivity with gelatine: perspective applications in tissue engineering. Biotechnol Lett 28(10):697–702

Birckbichler PJ, Patterson MK Jr (1978) Cellular transglutaminase, growth, and transformation. Ann N Y Acad Sci 312:354–365

Bowness JM, Folk JE, Timpl R (1987) Identification of a substrate site for liver transglutaminase on the aminopropeptide of type-III collagen. J Biol Chem 262(3):1022–1024

Bowness JM, Tarr AH, Wong T (1988) Increased transglutaminase activity during skin wound-healing in rats. Biochim Biophys Acta 967(2):234–240

Broderick EP, O'Halloran DM, Rochev YA, Griffin M, Collighan RJ, Pandit AS (2005) Enzymatic stabilization of gelatin-based scaffolds. J Biomed Mater Res B Appl Biomater 72B(1):37–42

Chau DYS, Collighan RJ, Griffin M (2007) Collagen: structure and modification for biomedical applications. In: Pannone P J (ed) Trends in biomaterials research. Nova Publishers, pp 143–190

Chau DYS, Collighan RJ, Verderio EAM, Addy VL, Griffin M (2005) The cellular response to transglutaminase-cross-linked collagen. Biomaterials 26(33):6518–6529

Chiono V, Ciardelli G, Vozzi G, Cortez J, Barbani N, Gentile P, Giusti P (2008) Enzymatically-modified melt-extruded guides for peripheral nerve repair. Eng Life Sci 8(3):226–237

Collier JH, Messersmith PB (2003) Enzymatic modification of self-assembled peptide structures with tissue transglutaminase. Bioconjugate Chem 14(4):748–755

Collighan R, Cortez J, Griffin M (2002) The biotechnological applications of transglutaminases. Minerva Biotechnol 14(2): 143–148

Collighan RJ, Li X, Parry J, Griffin M, Clara S (2004) Transglutaminases as tanning agents for the leather industry. J Am Leather Chem Ass 99(7):293–302

Condello S, Caccamo D, Curro M, Ferlazzo N, Parisi G, Ientile R (2008) Transglutaminase 2 and NF-kappa B interplay during NGF-induced differentiation of neuroblastoma cells. Brain Res 1207:1–8

Damodaran G, Collighan R, Griffin M, Pandit A (2008) Tethering a laminin peptide to a crosslinked collagen scaffold for biofunctionality. J Biomed Mater A 13:13

Dardik R, Krapp T, Rosenthal E, Loscalzo J, Inbal A (2007) Effect of FXIII on monocyte and fibroblast function. Cell Physiol Biochem 19(1–4):113–120

Dardik R, Loscalzo J, Inbal A (2006) Factor XIII (FXIII) and angiogenesis. J Thromb Haemost 4(1):19–25

Dolynchuk KN, Ziesmann M, Serletti JM (1996) Topical putrescine (Fibrostat) in treatment of hypertrophic scars: phase II study. Plast Reconstr Surg 97(1):117–123

Engler AJ, Sen S, Sweeney HL, Discher DE (2006) Matrix elasticity directs stem cell lineage specification. Cell 126(4):677–689

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

Fesus L, Szondy Z (2005) Transglutaminase 2 in the balance of cell death and survival. Febs Lett 579(15):3297–3302

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

Freund KF, Doshi KP, Gaul SL, Claremon DA, Remy DC, Baldwin JJ, Pitzenberger SM, Stern AM (1994) Transglutaminase inhibition by 2-(2-Oxopropyl)thio imidazolium derivative–mechanism of factor xiiia inactivation. Biochemistry 33(33): 10109–10119

Friess W (1998) Collagen—biomaterial for drug delivery. Eur J Pharm Biopharm 45(2):113–136

Fuchsbauer HL, Gerber U, Engelmann J, Seeger T, Sinks C, Hecht T (1996) Influence of gelatin matrices cross-linked with transglutaminase on the properties of an enclosed bioactive material using beta-galactosidase as model system. Biomaterials 17(15):1481–1488

Garcia Y, Collighan R, Griffin M, Pandit A (2007) Assessment of cell viability in a three-dimensional enzymatically cross-linked collagen scaffold. J Mater Sci Mater Med 18(10):1991–2001

Garcia Y, Wilkins B, Collighan RJ, Griffin M, Pandit A (2008) Towards development of a dermal rudiment for enhanced wound healing response. Biomaterials 29(7):857–868

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(43):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(1):104–113

Gentile V, Cooper AJ (2004) Transglutaminases—possible drug targets in human diseases. Curr Drug Targets CNS Neurol Disord 3(2):99–104

Goodman M, Cai WB, Kinberger GA (2003) The new science of protein mimetics. Macromol Sym 201:223–236

Grenard P, Bresson-Hadni S, El Alaoui S, Chevallier M, Vuitton DA, Ricard-Blum S (2001) Transglutaminase-mediated cross-linking is involved in the stabilization of extracellular matrix in human liver fibrosis. J Hepatol 35(3):367–375

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 IG, Rathbone DL (2008) Synthesis of potent water-soluble tissue transglutaminase inhibitors. Bioorg Med Chem Lett (in press)

Griffin M, Smith LL, Wynne J (1979) Changes in transglutaminase activity in an experimental model of pulmonary fibrosis induced by paraquat. Br J Exp Pathol 60(6):653–661

Griffin M, Wilson J (1984) Detection of epsilon(gamma-glutamyl) lysine. Mol Cell Biochem 58(1–2):37–49

Grigoriev MY, Suspitsin EN, Togo AV, Pozharisski KM, Ivanova OA, Nardacci R, Falasca L, Piacentini M, Imyanitov EN, Hanson KP (2001) Tissue transglutaminase expression in breast carcinomas. J Exp Clin Cancer Res 20(2):265–268

Gross SR, Balklava Z, Griffin M (2003) Importance of tissue transglutaminase in repair of extracellular matrices and cell death of dermal fibroblasts after exposure to a solarium ultraviolet a source. J Invest Dermatol 121(2):412–423

Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell 100(1):57–70

Hang J, Zemskov EA, Lorand L, Belkin AM (2005) Identification of a novel recognition sequence for fibronectin within the NH2-terminal beta-sandwich domain of tissue transglutaminase. J Biol Chem 280(25):23675–23683

Haroon ZA, Wannenburg T, Gupta M, Greenberg CS, Wallin R, Sane DC (2001) Localization of tissue transglutaminase in human carotid and coronary artery atherosclerosis: implications for plaque stability and progression. Lab Invest 81(1):83–93

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(Pt 3):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(6):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(21):3049–3058

Holmes TC (2002) Novel peptide-based biomaterial scaffolds for tissue engineering. Trends Biotechnol 20(1):16–21

Holmes TC, de Lacalle S, Su X, Liu G, Rich A, Zhang S (2000) Extensive neurite outgrowth and active synapse formation on self-assembling peptide scaffolds. Proc Natl Acad Sci USA 97(12):6728–6733

Hu BH, Messersmith PB (2003) Rational design of transglutaminase substrate peptides for rapid enzymatic formation of hydrogels. J Am Chem Soc 125(47):14298–14299

Hu BH, Messersmith PB (2005) Enzymatically cross-linked hydrogels and their adhesive strength to biosurfaces. Orthod Craniofac Res 8(3):145–149

Ichinose A, Bottenus RE, Davie EW (1990) Structure of transglutaminases. J Biol Chem 265(23):13411–13414

Isobe T, Takahashi H, Ueki S, Takagi J, Saito Y (1999) Activity-independent cell adhesion to tissue-type transglutaminase is mediated by alpha 4 beta 1 integrin. Eur J Cell Biol 78(12):876–883

Ito A, Mase A, Takizawa Y, Shinkai M, Honda H, Hata K, Ueda M, Kobayashi T (2003) Transglutaminase-mediated gelatin matrices incorporating cell adhesion factors as a biomaterial for tissue engineering. J Biosci Bioeng 95(2):196–199

Jelenska M, Fesus L, Kopec M (1980) The comparative ability of plasma and tissue transglutaminases to use collagen as a substrate. Biochim Biophys Acta 616(2):167–178

Johnson KA, Terkeltaub RA (2005) External GTP-bound transglutaminase 2 is a molecular switch for chondrocyte hypertrophic differentiation and calcification. J Biol Chem 280(15):15004–150012

Johnson TS, Fisher M, Haylor JL, Hau Z, Skill NJ, Jones R, Saint R, Coutts I, Vickers ME, El Nahas AM, Griffini M (2007) Transglutaminase inhibition reduces fibrosis and preserves function in experimental chronic kidney disease 18(12):3078–3088

Johnson TS, Griffin M, Thomas GL, Skill J, Cox A, Yang B, Nicholas B, Birckbichler PJ, Muchaneta-Kubara C, ElNahas AM (1997) The role of transglutaminase in the rat subtotal nephrectomy model of renal fibrosis. J Clin Invest 99(12):2950–2960

Johnson TS, Skill NJ, El Nahas AM, Oldroyd SD, Thomas GL, Douthwaite JA, Haylor JL, Griffin M (1999) Transglutaminase transcription and antigen translocation in experimental renal scarring. J Am Soc Nephrol 10(10):2146–2157

Jones MER, Messersmith PB (2007) Facile coupling of synthetic peptides and peptide-polymer conjugates to cartilage via transglutaminase enzyme. Biomaterials 28(35):5215–5224

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(9):1442–1453

Jurgensen K, Aeschlimann D, Cavin V, Genge M, Hunziker EB (1997) A new biological glue for cartilage-cartilage interfaces: tissue transglutaminase. J Bone Joint Surg-Am Vol 79A(2):185–193

Kaetsu H, Hashiguchi T, Foster D, Ichinose A (1996) Expression and release of the a and b subunits for human coagulation factor XIII in baby hamster kidney (BHK) cells. J Biochem (Tokyo) 119(5):961–969

Kawai Y, Wada F, Sugimura Y, Maki M, Hitomi K (2008) Transglutaminase 2 activity promotes membrane resealing after mechanical damage in the lung cancer cell line A549. Cell Biol Int 32(8):928–934

Khew ST, Yang QJ, Tong YW (2008) Enzymatically crosslinked collagen-mimetic dendrimers that promote integrin-targeted cell adhesion. Biomaterials 29(20):3034–3045

Kinberger GA, Cai W, Goodman M (2002) Collagen mimetic dendrimers. J Am Chem Soc 124(51):15162–15163

Kinberger GA, Taulane JP, Goodman M (2006) The design, synthesis, and characterization of a PAMAM-based triple helical collagen mimetic dendrimer. Tetrahedron 62(22):5280–5286

Kisiday J, Jin M, Kurz B, Hung H, Semino C, Zhang S, Grodzinsky AJ (2002) Self-assembling peptide hydrogel fosters chondrocyte extracellular matrix production and cell division: implications for cartilage tissue repair. Proc Natl Acad Sci USA 99(15):9996–10001

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(42):13768–13775

Knight CRL, Rees RC, Griffin M (1991) Apoptosis—a potential role for cytosolic transglutaminase and its importance in tumor progression. Biochim Biophys Acta 1096(4):312–318

Kotsakis P, Griffin M (2007) Tissue transglutaminase in tumour progression: friend or foe? Amino Acids 33(2):373–384

Lee KN, Arnold SA, Birckbichler PJ, Patterson MK, Fraij BM, Takeuchi Y, Carter HA (1993) Site-directed mutagenesis of human tissue transglutaminase: Cys-277 is essential for transglutaminase activity but not for GTPase activity. Biochim Biophys Acta 1202(1):1–6

Linge C, Richardson J, Vigor C, Clayton E, Hardas B, Rolfe KJ (2005) Hypertrophic scar cells fail to undergo a form of apoptosis specific to contractile collagen—the role of tissue transglutaminase. J Invest Dermatol 125(1):72–82

Liu SP, Cerione RA, Clardy J (2002) Structural basis for the guanine nucleotide-binding activity of tissue transglutaminase and its regulation of transamidation activity. Proc Natl Acad Sci USA 99(5):2743–2747

Mangala LS, Arun B, Sahin AA, Mehta K (2005) Tissue transglutaminase-induced alterations in extracellular matrix inhibit tumor invasion. Mol Cancer 4:33

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(17):2459–2470

Mann AP, Verma A, Sethi G, Manavathi B, Wang HM, Fok JY, Kunnumakkara AB, Kumar R, Aggarwal BB, Mehta K (2006) Overexpression of tissue transglutaminase leads to constitutive activation of nuclear factor-kappa B in cancer cells: delineation of a novel pathway. Cancer Res 66(17):8788–8795

McHale MK, Setton LA, Chilkoti A (2005) Synthesis and in vitro evaluation of enzymatically cross-linked elastin-like polypeptide gels for cartilaginous tissue repair. Tissue Eng 11(11–12):1768–1779

Mehta K (1994) High levels of transglutaminase expression in doxorubicin-resistant human breast carcinoma cells. Int J Cancer 58(3):400–406

Mian S, el Alaoui S, Lawry J, Gentile V, Davies PJ, Griffin M (1995) The importance of the GTP-binding protein tissue transglutaminase in the regulation of cell cycle progression. Febs Lett 370(1–2):27–31

Mirza A, Liu SL, Frizell E, Zhu JL, Maddukuri S, Martinez J, Davies P, Schwarting R, Norton P, Zern MA (1997) A role for tissue transglutaminase in hepatic injury and fibrogenesis, and its regulation by NF-kappa B. Am J Physiol-Gastroint Liver Physiol 35(2):G281–G288

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(23):23863–23868

Nicholas B, Smethurst P, Verderio E, Jones R, Griffin M (2003) Cross-linking of cellular proteins by tissue transglutaminase during necrotic cell death: a mechanism for maintaining tissue integrity. Biochem J 371:413–422

O'Halloran DM, Russell JC, Griffin M, Pandit AS (2006) Characterization of a microbial transglutaminase cross-linked type II collagen scaffold. Tissue Eng 12(6):1467–1474

O'Halloran D, Grad S, Stoddart M, Dockery P, Alini M, Pandit AS (2008) An injectable cross-linked scaffold for nucleus pulposus regeneration. Biomaterials 29(4):438–447

Oliverio S, Amendola A, DiSano F, Farrace MG, Fesus L, Nemes Z, Piredda L, Spinedi A, Piacentini M (1997) Tissue transglutaminase-dependent posttranslational modification of the retinoblastoma gene product in promonocytic cells undergoing apoptosis. Mol Cell Biol 17(10):6040–6048

Orban JM, Wilson LB, Kofroth JA, El-Kurdi MS, Maul TM, Vorp DA (2004) Crosslinking of collagen gels by transglutaminase. J Biomed Mater A 68A(4):756–762

Piacentini M, Piredda L, Starace D, AnnicchiaricoPetruzzelli M, Mattei M, Oliverio S, Farrace MG, Melino G (1996) Differential growth of N- and S-type human neuroblastoma cells xenografted into SCID mice—correlation with apoptosis. J Pathol 180(4):415–422

Pinkas DM, Strop P, Brunger AT, Khosla C (2007) Transglutaminase 2 undergoes a large conformational change upon activation. Plos Biology 5(12):2788–2796

Raghunath M, Hopfner B, Aeschlimann D, Luthi U, Meuli M, Altermatt S, Gobet R, BrucknerTuderman L, Steinmann B (1996) Cross-linking of the dermo-epidermal junction of skin regenerating from keratinocyte autografts—anchoring fibrils are

a target for tissue transglutaminase. J Clin Invest 98(5):1174–1184

Richards RJ, Masek LC, Brown RFR (1991) Biochemical and cellular mechanisms of pulmonary fibrosis. Toxicol Pathol 19(4):526–539

Shweke N, Boulos N, Jouanneau C, Vandermeersch S, Melino G, Dussaule JC, Chatziantoniou C, Ronco P, Boffa JJ (2008) Tissue transglutaminase contributes to interstitial renal fibrosis by favoring accumulation of fibrillar collagen through TGF-beta activation and cell infiltration. Am J Pathol 173(3):631–642

Skill NJ, Griffin M, El Nahas AM, Sanai T, Haylor JL, Fisher M, Jamie MF, Mould NN, Johnson TS (2001) Increases in renal epsilon-(gamma-glutamyl)-lysine crosslinks result from compartment-specific changes in tissue transglutaminase in early experimental diabetic nephropathy: pathologic implications. Lab Invest 81(5):705–716

Sperinde JJ, Griffith LG (1997) Synthesis and characterization of enzymatically-cross-linked poly(ethylene glycol) hydrogels. Macromolecules 30(18):5255–5264

Stenberg P, Roth EB, Sjoberg K (2008) Transglutaminase and the pathogenesis of coeliac disease. Eur J Intern Med 19(2):83–91

Summey BT, Graff RD, Lai TS, Greenberg CS, Lee GM (2002) Tissue transglutaminase localization and activity regulation in the extracellular matrix of articular cartilage. J Orthop Res 20(1):76–82

Takahashi H, Isobe T, Horibe S, Takagi J, Yokosaki Y, Sheppard D, Saito Y (2000) Tissue transglutaminase, coagulation factor XIII, and the pro- polypeptide of von Willebrand factor are all ligands for the integrins alpha(9)beta(1) and alpha(4)beta(1). J Biol Chem 275(31):23589–23595

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(6):409–418

Telci D, Griffin M (2006) Tissue transglutaminase (TG2)—a wound response enzyme. Front Biosci 11:867–882

Telci D, Wang Z, Li X, Verderio EAM, Humphries MJ, Baccarini M, Basaga H, Griffin M (2008) Fibronectin-tissue transglutaminase matrix rescues RGD-impaired cell adhesion through syndecan-4 and beta(1) integrin co-signaling. J Biol Chem 283(30):20937–20947

Tovar-Vidales T, Roque R, Clark AF, Wordinger RJ (2008) Tissue transglutaminase expression and activity in normal and glaucomatous human trabecular meshwork cells and tissues. Ophthalmol Vis Sci 49(2):622–628

Ueki S, Takagi J, Saito Y (1996) Dual functions of transglutaminase in novel cell adhesion. J Cell Sci 109(Pt 11):2727–2735

Upchurch HF, Conway E, Patterson MK Jr, Birckbichler PJ, Maxwell MD (1987) Cellular transglutaminase has affinity for extracellular matrix. In Vitro Cell Dev Biol 23(11):795–800

Upchurch HF, Conway E, Patterson MK, Maxwell MD (1991) Localization of cellular transglutaminase on the extracellular matrix after wounding—characteristics of the matrix bound enzyme. J Cell Physiol 149(3):375–382

Verderio E, Coombes A, Jones RA, Li XL, Heath D, Downes S, Griffin M (2001) Role of the cross-linking enzyme tissue transglutaminase in the biological recognition of synthetic biodegradable polymers. J Biomed Mater Res 54(2):294–304

Verderio E, Gross SR, Griffin M (1999) Cell-surface tissue transglutaminase regulates matrix storage of latent TGF-beta binding protein-1 (LTBP-1) and fibronectin accumulation. Mol Biol Cell 10:370

Verderio EA, Scarpellini A and Johnson TS (2008) Novel interactions of TG2 with heparan sulfate proteoglycans: reflection on physiological implications. Amino Acids (in press)

Verderio EAM, Johnson TS, Griffin M (2005) Transglutaminases in wound healing and inflammation. Prog Exp Tumor Res 38:89–114

Verderio EAM, 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(43):42604–42614

Weiss MS, Metzner HJ, Hilgenfeld R (1998) Two non-proline cis peptide bonds may be important for factor XIII function. Febs Lett 423(3):291–296

Welge-Lussen U, May CA, Lutjen-Drecoll E (2000) Induction of tissue transglutaminase in the trabecular meshwork by TGF-beta 1 and TGF-beta 2. Invest Ophthalmol Vis Sci 41(8):2229–2238

Wilhelmus M, Andringa G, Bol J, Van Dam AM, Hoozemans J, Jongenelen C, Breve J, Boekel A, Drukarch B (2007) Tissue transglutaminase is activated and redistributed to ER-derived granules in neuroblastoma cells treated with the PD-mimicking neurotoxin MPP. Park Rel Dis 13:S135–S135

Zemskov EA, Janiak A, Hang J, Waghray A, Belkin AM (2006) The role of tissue transglutaminase in cell-matrix interactions. Front Biosci 11:1057–1076

Zemskov EA, Mikhailenko I, Strickland DK, Belkin AM (2007) Cell-surface transglutaminase undergoes internalization and lysosomal degradation: an essential role for LRP1. J Cell Sci 120(18):3188–3199

Zhu Y, Tramper J (2008) Novel applications for microbial transglutaminase beyond food processing. Trends Biotechnol 26(10):559–565