Amino Acids (2010) 39:619–631
DOI 10.1007/s00726-010-0500-z

REVIEW ARTICLE

Transglutaminase 2: a multi-functional protein in multiple subcellular compartments

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Received: 9 November 2009 / Accepted: 23 January 2010 / Published online: 11 February 2010

Abstract Transglutaminase 2 (TG2) is a multifunctional protein that can function as a transglutaminase, G protein, kinase, protein disulfide isomerase, and as an adaptor protein. These multiple biochemical activities of TG2 account for, at least in part, its involvement in a wide variety of cellular processes encompassing differentiation, cell death, inflammation, cell migration, and wound healing. The individual biochemical activities of TG2 are regulated by several cellular factors, including calcium, nucleotides, and redox potential, which vary depending on its subcellular location. Thus, the microenvironments of the subcellular compartments to which TG2 localizes, such as the cytosol, plasma membrane, nucleus, mitochondria, or extracellular space, are important determinants to switch on or off various TG2 biochemical activities. Furthermore, TG2 interacts with a distinct subset of proteins and/or substrates depending on its subcellular location. In this review, the biological functions and molecular interactions of TG2 will be discussed in the context of the unique environments of the subcellular compartments to which TG2 localizes.

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Keywords TG2 · Subcellular localization · Subcellular compartment · Organelle · Transglutaminase · G_h · Protein disulfide isomerase · Kinase

Introduction

Transglutaminase 2 (TG2), also known as tissue transglutaminase or transglutaminase C, is a member of the transglutaminase (TGase) family. TG2 catalyzes protein cross-linking via transamidation of glutamine residues to lysine residues in a Ca^{2+}-dependent manner (Lorand and Graham 2003; Facchiano and Facchiano 2009). The cross-linking generates proteolytically resistant γ-glutamyl-ε-lysine bonds. The resulting cross-linked proteins often have high-molecular masses. Besides its classical protein cross-linking activity, TG2 possesses several other biochemical functions. TG2 can function as a G protein (high-molecular weight GTP-binding protein), designated G_h, by mediating α1-adrenergic receptor signaling in rat liver membranes (Nakaoka et al. 1994). TG2 also has protein disulfide isomerase (PDI) (Hasegawa et al. 2003), protein kinase (Mishra and Murphy 2004; Mishra et al. 2007), and DNA nuclease activities (Takeuchi et al. 1998). In addition to its various enzymatic activities, TG2 interacts directly with fibronectin, integrins, and syndecan, therefore, mediating cell-extracellular matrix (ECM) interactions (Telci et al. 2008; Collighan and Griffin 2009).

This multifunctional protein is expressed ubiquitously and abundantly, and is implicated in a variety of cellular processes, such as differentiation, cell death, inflammation, cell migration, and wound healing (reviewed in Fesus and Piacentini 2002; Lorand and Graham 2003; Fesus and Szondy 2005; Ientile et al. 2007; Collighan and Griffin 2009; Sarang et al. 2009). Beyond its functional diversity,

TG2 has both pro- and anti-apoptotic functions (Fesus and Szondy 2005). To reconcile seemingly contradictory sets of observations, several parameters have been considered (Fesus and Szondy 2005), including cell types (Shin et al. 2004), the type of cell death inducers (Tucholski and Johnson 2002), the intensity of a stimulus (Jeong et al. 2009), and TG2-splice variants (Monsonego et al. 1997; Antonyak et al. 2006). Subcellular localization of TG2 was suggested as another important determinant of its functions because targeting TG2 to different compartments resulted in different effects on apoptosis (Milakovic et al. 2004). With a general acceptance of the idea that TGase and GTPase activities are modulated by the local environment (Lai et al. 1998), TG2 is believed to create distinct molecular interactions in different subcellular compartments, which in turn exert diverse effects on cellular physiology.

TG2 Structure and regulation by small molecules

Human TG2 has been crystallized in two different conformations with GDP or a pentapeptide inhibitor (Liu et al. 2002; Pinkas et al. 2007). Although the two structures represent the two extremes of movement, both structures clearly showed that the monomer has four distinct domains: an N-terminal β-sandwich domain, a transamidation catalytic core, and two C-terminal barrels (Liu et al. 2002; Pinkas et al. 2007). Three-dimensional structures of TG2 suggest a molecular basis for reciprocal regulation by calcium and nucleotides (ATP and GTP), key regulators of various enzymatic activities of TG2 (Table 1). Calcium activates TGase activity by inducing a conformational change that increases the interdomain distance between the catalytic domain and the two C-terminal barrel domains, consequently exposing the active site of the TGase to the substrate (Pinkas et al. 2007). Calcium is either inhibitory or indispensible for the G_h, PDI, and kinase activities of TG2, whereas calcium is essential for the TGase activity (Table 1; Fig. 1). In contrast, GTP binding likely stabilizes the closed conformation, therefore negatively regulating the TGase activity and positively regulating the G_h activity of TG2 (Nakaoka et al. 1994; Iismaa et al. 2000; Begg et al. 2006b). In addition to its GTPase activity, TG2 also hydrolyzes ATP in a site distinct from that of GTP hydrolysis (Lai et al. 1998). Mg-ATP binds TG2 at a 3-fold greater steady-state affinity than Mg-GTP and is hydrolyzed at a similar rate (Lai et al. 1998). In contrast to Mg-GTP, Mg-ATP does not inhibit the TGase activity, but rather inhibits GTP hydrolysis (Lai et al. 1998). Inhibition of the GTPase activity by ATP, if GTP binding is not affected, could potentiate the G_h activity (Johnson and Terkeltaub 2005). In addition to calcium and nucleotides, sphingosylphosphocholine (lyso-SM) and nitric oxide are able to modulate TG2 TGase activity (Lai et al. 1997; Bernassola et al. 1999; Rossi et al. 2000). The presence of lyso-SM reduced the calcium concentration required for TGase activation from 10 to 160 μM (Lai et al. 1997). On the other hand, nitric oxide inhibited TG2 TGase activity via chemical modification, possibly *S*-nitrosylation, of a cysteine residue (Bernassola et al. 1999; Rossi et al. 2000).

The PDI activity of TG2 did not require calcium and was greatly modulated by concentrations of oxidants and antioxidants (Table 1) (Hasegawa et al. 2003). The PDI activity was potentiated by the presence of glutathione disulfide (GSSG, 10 μM), which was not substantially inhibited by glutathione (GSH), even at high levels (approximately

Table 1 Positive (+) or negative (−) regulation of TG2 enzymatic activities by small molecules

	TGase	G_h	PDI	Kinase
Calcium	+ (Takeuchi et al. 1992)	− (Achyuthan and Greenberg 1987)	−[a] (Hasegawa et al. 2003)	− (Mishra and Murphy 2004; Mishra et al. 2007)
GTP	− (Achyuthan and Greenberg 1987; Nakaoka et al. 1994)	+ (Nakaoka et al. 1994)	ns (Hasegawa et al. 2003)	nd
ATP	ns (Lai et al. 1998)	+ (Johnson and Terkeltaub 2005)	ns (Hasegawa et al. 2003)	+ (Mishra et al. 2007)
Lyso-SM	+ (Lai et al. 1997)	nd	nd	nd
Reducing agents	+ (Verderio et al. 2003)	nd	− (Hasegawa et al. 2003)	nd
C277S	− (Lee et al. 1993; Chen et al. 1996)	ns (Lee et al. 1993; Chen et al. 1996)	nd	nd
TGase inhibitors	− (MDC) (Davies et al. 1980)	ns	ns (putrescine) (Hasegawa et al. 2003)	− (MDC, cystamine) (Mishra and Murphy 2004)

[a] In the presence of calcium, substrates of the PDI became unavailable due to its TGase activity (Hasegawa et al. 2003). C277S is a TG2 mutant in which Cys 277 is replaced by Ser. TGase inhibitors in parentheses have been examined for their effects on corresponding TG2 enzymatic activities

Lyso-SM sphingosylphosphocholine, *ns* not significant, *nd* not determined, *MDC* monodansylcadaverine

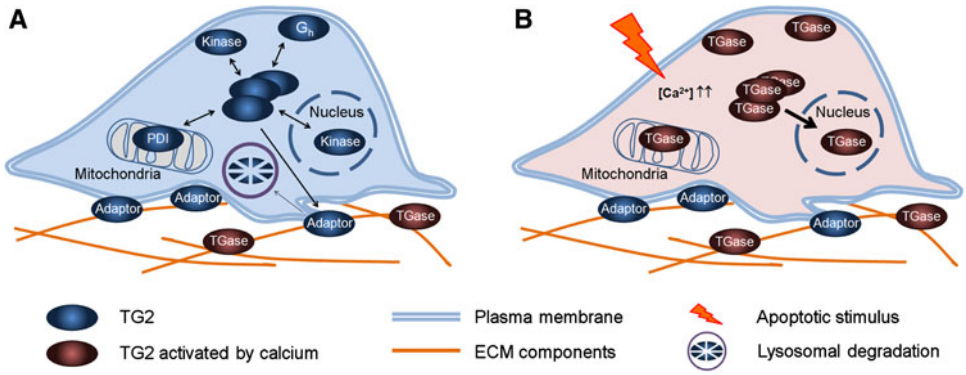

Fig. 1 Depending on its subcellular localization, TG2 presents various biochemical activities, such as a transglutaminase (TGase), G protein (G_h), kinase, protein disulfide isomerase (PDI), and/or as an adaptor protein. **a** At low calcium concentrations under normal physiological conditions, intracellular TG2 does not display TGase activity, but rather other activities including G_h, kinase, and PDI. These TG2 enzymatic activities are found in mitochondria, the nucleus, and on the plasma membrane. Extracellular TG2 functions in cell adhesion as an adaptor protein and in ECM remodeling via TGase activity. **b** In response to apoptotic stimuli, elevated calcium concentrations potentiate the TGase activity and suppress all other activities. TG2 TGase catalyzes massive intracellular protein cross-links, generating protective shells that reduce the leakage of intracellular contents of apoptotic cells. During the process, the activated TGase is translocated to the nucleus, which might mediate chromatin condensation. Intracellular calcium levels are indicated by background color; low concentrations are represented by a shade of *blue* and high values by *red*

500 μM). These data suggest that TG2 PDI activity was present in the fairly reducing cytoplasmic environment (Hasegawa et al. 2003). Although cytosolic proteins do not usually have disulfide bonds due to the reducing nature of the environment, TG2 was estimated to have five to six disulfide bonds (Hasegawa et al. 2003). However, it is not clear which of these disulfide bonds is involved in the reaction.

In addition to these small molecules, more complicated cellular signaling might be involved in regulating TG2 activities. In particular, protein kinase A was shown to phosphorylate TG2, attenuating its TGase activity and enhancing its kinase activity (Mishra et al. 2007). Thus, variations of these regulators across various subcellular compartments would likely influence the biochemical activities of TG2.

TG2 at various subcellular locations

TG2 is predominantly a cytoplasmic protein, but it is also found in the nucleus and mitochondria, on the plasma membrane and the extracellular cell surface, and in the extracellular matrix (ECM). TG2 has been shown to dynamically translocate depending on the state of cell proliferation or in response to elevation of intracellular calcium concentrations (Korner et al. 1989; Peng et al. 1999). Although several factors have been proposed to be involved in TG2 translocation, the underlying mechanisms by which TG2 is translocated to various subcellular compartments remains unclear. Nonetheless, substantial evidence indicates that the subcellular location of TG2 is critical for regulation of its various biochemical activities, which subsequently trigger diverse downstream events.

Cytoplasm

The cytoplasmic environment

TG2 TGase activity is usually suppressed in cytoplasmic environments due to low intracellular calcium concentrations and high GTP concentrations. The intracellular free GTP concentration has been estimated to be approximately 100–150 μM (Horie and Irisawa 1989; Hatakeyama et al. 1992), which should inhibit the TGase. Mg-GTP inhibited the TGase activity in a reaction containing 1 mM calcium with an IC_{50} value of 9 μM (Lai et al. 1998). A study using electro-permeabilized human endothelial cells indicated that intracellular TGase activity is virtually zero at physiological concentrations of ATP and GTP, even in the presence of 10 μM calcium (Smethurst and Griffin 1996). Consistently, depletion of GTP in situ resulted in a significant increase in TGase activity (Zhang et al. 1998; Lesort et al. 2000b). Thus, most of TG2 TGase-substrates have been identified under conditions that artificially activate the TGase.

Leaving experimental conditions aside, the majority of TG2 TGase-substrates identified so far are found in the cytoplasm, which is in concordance with the fact that TG2 is predominantly a cytoplasmic protein. Approximately two thirds of the intracellular proteins identified as TGase substrates in the Transdab wiki database (Csosz et al. 2009) were known to be primarily located in the cytoplasm.

Activation of TGase activity

TGase activity is induced by various stressful and/or apoptotic stimuli, such as maitotoxin, calphostin C, UV

irradiation, and oxidative stress (Lesort et al. 1998; Hebert et al. 2000; Lee et al. 2003; Robitaille et al. 2004; Shin et al. 2004; Yi et al. 2004; Ientile et al. 2007). In particular, numerous studies have focused on activation of TG2 by reactive oxygen species (ROS). ROS levels are dramatically increased by environmental stresses, such as UV or heat exposure, which may result in cell death via direct and/or indirect consequences. Previously, we showed that intracellular ROS was essential for the activation of in situ TGase in response to lysophosphatidic acid (LPA) and transforming growth factor-β (TGF-β) in Swiss 3T3 fibroblast cells (Lee et al. 2003). Intracellular ROS also mediated the TGase activation by arachidonic acid in NIH3T3 cells (Yi et al. 2004). The role of ROS in the activation of the TGase was supported by the in situ activation of TG2 by exogenous H_2O_2 (Lee et al. 2003; Yi et al. 2004). However, exogenous H_2O_2 did not potentiate in vitro TGase activity (data not shown). This indicates that in situ TG2 is indirectly activated by accumulation of intracellular ROS, which is likely due to elevation of intracellular calcium.

Diverse effects of TG2 on the cell death decision

Upon its activation by various apoptotic stimuli, TG2 has been reported to play various roles in cell death. Cytosolic TG2 has been implicated in exerting anti-apoptotic effects via activation of the NF-κB pathway in several cancer cell lines (Mann et al. 2006; Cao et al. 2008; Condello et al. 2008) or polymerization of cytosolic nucleophosmin in MCF7 cells (Park et al. 2009). In contrast, there is evidence that suggests a pro-apoptotic effect of TG2. Inhibition of TG2 expression prevented death in U937 cells upon apoptotic stimulus (Oliverio et al. 1999). It has been suggested that in NIH3T3 cells treated with calphositin C, TG2 induced apoptosis by cross-linking of a dual leucine zipper-bearing kinase (DLK), a pro-apoptotic kinase (Robitaille et al. 2004, 2008). TG2 facilitated calcium-induced apoptotic cell death via an apoptosis-inducing factor-dependent manner in a pancreatic cancer cell line, Panc-28 cells (Fok and Mehta 2007). Thus, depending on several parameters, such as cell type and apoptotic stimuli, TG2 can exert quite diverse downstream effects resulting in cell death or survival.

TG2 TGase activity during apoptotic cell death

Once cells undergo apoptotic cell death, TG2 is activated to catalyze protein cross-linking, resulting in protective shells that prevent the leakage of harmful cell contents from the apoptotic cells (Piredda et al. 1997; Nicholas et al. 2003). Cross-linking fibrous proteins is an efficient way to generate such protective shells. Indeed, cytoskeletal proteins and their associated proteins have been shown to be substrates of TG2 when intracellular levels of calcium were increased by the calcium ionophore A-23187 (Nemes et al. 1997; Orru et al. 2003). It has been proposed that the cross-linked protein-rich shells prevent leakage of harmful cell contents from apoptotic cells, facilitate apoptotic cell death and removal of dead cells, and consequently lessen inflammation and apparent tissue damage (Fesus and Szondy 2005). The lack of TG2 in MRLlpr/lpr mice was suggested to contribute to the development of autoimmunity due to large amounts of circulating TG2 autoantibody (Piredda et al. 1997). Overexpression of TG2 prevented the release of macromolecules, such as DNA and lactate dehydrogenase, when L929 cells were killed by tumor necrosis factor-α (TNF-α) (Piredda et al. 1997). These observations are in accordance with results obtained from TG2 knockout mice. For a review on a wide variety of phenotypic defects in TG2−/− mice, refer to (Sarang et al. 2009). When apoptosis was induced in the thymus or liver of TG2−/− mice, the clearance of apoptotic cells was delayed, indicating that TG2 is required for efficient phagocytosis of apoptotic bodies (Szondy et al. 2003). The defective clearance of apoptotic cells was accompanied by an inflammatory reaction in the liver of TG2−/− mice (Szondy et al. 2003). TG2−/− red blood cells (RBCs) were engulfed more slowly by wild-type macrophages, suggesting that TG2 is required for phagocytosis of apoptotic RBCs (Sarang et al. 2007). Studies of TG2−/− mice suggest that TG2 facilitates phagocytosis, not only in apoptotic cells, but also in macrophages (Falasca et al. 2005; Rebe et al. 2009). For example, macrophages isolated from TG2−/− mice had impaired capacities for engulfing apoptotic cells (Falasca et al. 2005). Thus, the evidence coherently suggests that TG2 contributes to the clearance of apoptotic cells, and to the reduction of leakage of intracellular contents into tissues.

Plasma membrane

TG2 as a G protein (G_h) on the plasma membrane

When associated with the plasma membrane, TG2 functions as a G protein, designated G_h, by mediating receptor signaling. G_h was shown to mediate α_1-adrenergic receptor (α_1-AR) signaling in rat liver membranes (Nakaoka et al. 1994), as the signaling was absent in TG2−/− mice (Nanda et al. 2001). The absence of TG2 prevented (-)epinephrine, an α_1-AR agonist, from stimulating [α-^{32}P]GTP-photolabeling of a 74-kDa protein in the liver (Nanda et al. 2001). Agonist activation of TPα thromboxane A2 receptors induced G_h stimulation of phospholipase C (PLC)-mediated inositol phosphate (IP) production (Vezza et al. 1999).

There have been contradictory observations suggesting different underlying mechanisms whereby G_h triggers downstream events, such as activation of IP production. Feng et al. (1996) demonstrated that the physical interaction of G_h with PLC δ1 resulted in activation of the latter. In contrast, Murthy et al. (1999) proposed an inhibitory model, where PLC δ1 is suppressed by a physical interaction with TG2. Cytosolic TG2 was suggested to inhibit PLC δ1 by forming a PLC δ1:TG2 complex, whereas binding of TG2 with GTP released PLC δ1 from the inhibitory association (Murthy et al. 1999). On the other hand, it was also suggested that PLC δ1 plays a role as a guanine nucleotide exchanging factor (GEF) for TG2 and stabilizes the GTP:TG2 complex, implying a reciprocal regulation (Baek et al. 2001). Thus, further investigation is required to understand how G_h activity triggers downstream signaling pathways.

Functions of TG2 on the plasma membrane

Several studies indicate that G_h activity contributes to cell survival. A study analyzing TG2 knockout mice showed that G_h is involved in the liver regeneration program via the α1-adrenergic receptor signaling pathway (Broten et al. 1999; Wu et al. 2000; Sarang et al. 2005). G_h activity of TG2 has been associated with the cell survival effect in NIH3T3 cells (Antonyak et al. 2002; Antonyak et al. 2003). A study that examined a number of point mutants in human TG2 suggested that the loss of GTP-binding activity can convert cytoprotective TG2 into a cell death-promoting factor (Datta et al. 2007).

During association with the plasma membrane, both the G_h and TGase activities of TG2 have been suggested to play a role in cell migration. In human aortic smooth muscle cells or human embryonic kidney cells, G_h was shown to modulate cell motility via its interaction with integrins (Kang et al. 2004). TG2 was co-immunoprecipitated with integrin-α_5 and was bound to the cytoplasmic tails of integrins in the presence of GTPγS (Kang et al. 2004). Treatment of HeLa cells with epidermal growth factor (EGF) resulted in TGase activation and its accumulation on the plasma membrane. This was particularly apparent at the leading edges of migratory cells where the TGase plays a critical role in EGF-stimulated cell migration and invasion (Antonyak et al. 2009). Ras and JNK were shown to mediate downstream signaling of EGF, causing TG2 to localize to the leading edges of cells and become an active TGase (Antonyak et al. 2009).

TG2 on the plasma membrane differs from cytosolic TG2

From a biochemical perspective, TG2 on the plasma membrane appears to be different from cytoplasmic TG2. Retinoic acid (RA)-treatment of HeLa cells enhanced GTP binding of TG2 and promoted the association of TG2 with membrane fractions (Singh and Cerione 1996). Using a gel filtration column, TG2 was predominantly eluted in an approximately 600-kDa complex in control cells, whereas TG2 was also found in an additional 150-kDa complex following RA treatment (Singh and Cerione 1996). Taken together, it was proposed that, upon treatment with RA, TG2 dissociated from a large molecular mass complex and bound to the plasma membrane (Singh and Cerione 1996). Although the structural configurations in both compartments are not clear, the biochemical activities of TG2 in the two types of complexes appear to be different. TG2 found in the 150-kDa complex showed GTP-binding, GTP-hydrolytic, and transglutaminase activities, whereas TG2 found in the 600-kDa complex had none of these activities (Singh and Cerione 1996). Simultaneous activation of both GTPase and TGase activity in the 150-kDa complex is somewhat perplexing because the TGase and G_h activities of TG2 are inversely regulated by calcium and GTP (Achyuthan and Greenberg 1987; Nakaoka et al. 1994; Iismaa et al. 2000; Begg et al. 2006a, b). Thus, TG2 activities in those complexes are likely regulated by additional cellular factors that differ between the two subcellular compartments. In this regard, it is intriguing that TG2 can be activated at a relatively low calcium concentration in the presence of lyso-SM (Lai et al. 1997). Later, it was suggested that β-tubulin, GST p1-1, and an unknown 110-kDa protein form a large complex with TG2 in SK-N-BE cells (Piredda et al. 1999), although it is not clear if these interactions play important roles in the regulation of various TG2 activities.

Recently, TG2 isolated from membrane fractions was found to phosphorylate insulin-like growth factor-binding protein-3 (IGFBP-3) (Mishra and Murphy 2004). Although the physiological significance of TG2 kinase activity remains to be evaluated, it would be worthwhile to examine whether the kinase activity of TG2 is also modulated by its translocation between the cytoplasm and the plasma membrane.

Nucleus

Localization of TG2 in the nucleus

The presence of TG2 in the nucleus has been reported in various cell types, including rabbit liver nuclear preparations (Singh et al. 1995). Additionally, subcellular fractionation of human neuroblastoma SH-SY5Y cells showed that 7% of recovered TG2 was found in the nucleus (Lesort et al. 1998). When intracellular calcium levels increase, the active TGase translocates to the nucleus (Lesort et al. 1998; Peng et al. 1999; Campisi et al. 2003; Mann et al. 2006).

Lesort et al. (1998) demonstrated that TG2 translocated to the nucleus in response to elevation of intracellular calcium levels by maitotoxin. Glutamate promoted TG2 translocation to the nuclear compartment in differentiated astrocytes (Campisi et al. 2003). Treatment of Panc28 pancreatic cancer cells with the calcium ionophore A-23187 induced translocation of TG2 into the nucleus (Mann et al. 2006). As observed by electron microscopy, RA induced translocation of TG2 to the nucleus in a non-small cell lung cancer cell line (Peng et al. 1999).

Although TG2 translocation to the nucleus is poorly understood at the molecular level, importin-α3, a nuclear transport protein, was suggested as a mediator of active TG2 nuclear transport (Peng et al. 1999). Yeast two-hybrid and co-immunoprecipitation assays demonstrated an interaction between importin-α3 and TG2 (Peng et al. 1999). A putative bipartite nuclear localization sequence (NLS), with high homology to the NLS of the influenza virus non-structural protein NS1, was identified in the primary sequence of TG2 (Peng et al. 1999). However, the functional significance of the NLS of TG2 and the interaction of TG2 with importin-α3 are still unclear.

Molecular interactions of TG2 in the nucleus

Several nuclear proteins have been identified as potential substrates of the TGase. Retinoblastoma (Rb) protein, an important regulator of apoptosis, was identified as a potential substrate in U937 cells undergoing apoptosis (Oliverio et al. 1997). The TGase was subsequently reported to protect Rb from caspase-induced degradation in a transamidation-dependent manner. This was suggested to provide an anti-apoptotic effect against all-*trans*-*N*-(4-hydroxyphenyl)retinamide (HPR) (Boehm et al. 2002). However, the role of the TGase activity in the anti-apoptotic effect was somewhat contradictory to the previous result in that the G_h activity of TG2 was sufficient to protect HL60 cells from HPR-induced death (Antonyak et al. 2001). Moreover, Milakovic et al. (2004) showed that TG2 does not require its TGase activity to protect HEK293 cells against thapsigargin-induced apoptosis. C277S TG2, a mutant that lacks transamidating activity, has a protective effect against apoptosis when targeted to the nucleus (Milakovic et al. 2004). The anti-apoptotic effect correlated with increased interactions with Rb, but protection of Rb from degradation by cross-linking was not observed. Although further investigation is necessary to reconcile this discrepancy, TG2 might exert similar anti-apoptotic effects through different mechanisms depending on the cell type and the type or intensity of the cell death inducer.

Recently, Rb was shown to be a substrate for TG2 kinase activity (Mishra et al. 2007). The study by Mishra et al. raised an intriguing possibility that the anti-apoptotic effect of C277S TG2 might be mediated by phosphorylation of Rb by TG2. However, it still needs to be tested whether the C277S TG2 mutant maintains kinase activity. Besides Rb, p53 and histones were shown to be phosphorylated by TG2 (Mishra and Murphy 2006; Mishra et al. 2007). TG2 is able to not only phosphorylate histones, but also cross-link histones in vitro. This suggests that TG2 might be involved in the epigenetic regulation of chromatin structure and function via posttranslational modifications of histone proteins (Ballestar et al. 1996, 2001; Kim et al. 2001; Mishra et al. 2006). Histone cross-linking catalyzed by TG2 has also been proposed to mediate chromatin condensation, a morphological hallmark of apoptosis (Ballestar et al. 1996). In the nucleus, TG2 might have DNA nuclease activity during apoptotic cell death. Sphingosine treatment of human melanocytic A375-S2 cells induced TG2 translocation from the cytoplasm to the nuclei, where TG2 mediated DNA hydrolysis during apoptotic cell death (Takeuchi et al. 1998). However, this finding should be verified by independent research groups.

Mitochondria

Mitochondrial environment

Mitochondria consist of four subcompartments: the outer mitochondrial membrane (OMM), the inner membrane space (IMS), the inner mitochondrial membrane (IMM), and the mitochondrial matrix. Small molecules up to 3 kDa are able to diffuse freely between the cytoplasm and the IMS through 2-nm aqueous pores in the OMM (Benz 1990). Therefore, calcium and nucleotide concentrations in the IMS may not differ significantly from those in the cytoplasm. In contrast, passive diffusion of any molecules through the IMM is very difficult. Thus, the IMM forms a barrier between the cytoplasm and the mitochondrial matrix. Since the mitochondrial matrix is a significant storage site of calcium in the cell, free calcium concentrations in the mitochondrial matrix are normally kept much higher (1–5 μM) than in the cytoplasm (Chalmers and Nicholls 2003). Calcium at this range of concentrations may not be high enough to activate the TGase activity in the presence of GTP under normal physiological conditions. However, intracellular calcium overload is likely to lead to TGase activation in the IMS or mitochondrial matrix.

Localization of TG2 in mitochondria

Our analysis of the amino-terminal sequence of TG2 did not reveal an obvious signal sequence containing the targeting information for mitochondrial precursor proteins (data not shown). Although it is not a typical mitochondrial

protein, TG2 seems to play an important role in mitochondria. Given the important roles of mitochondria in regulating apoptosis, the various effects of TG2 on apoptotic cell death led to investigation of the involvement of TG2 in mitochondrial functions. In SK-n-BE(2) cells, approximately half of the TG2 was associated with mitochondria (Rodolfo et al. 2004). Upon cell death induced by staurosporine (STS) treatment, TG2 cross-linked Bax on the OMM, which may result in the stabilization of the Bax oligomer, and consequently a stably open pore in the OMM (Rodolfo et al. 2004). Since expression of TG2 in neuroblastoma and 3T3 fibroblast cells resulted in hyperpolarization of mitochondria and increased ROS production (Piacentini et al. 2002), localization of TG2 in mitochondria might not be limited to the OMM. Moreover, several mitochondrial matrix proteins have been shown to be TGase substrates, including aconitase (Kim et al. 2005), Hsp70/90 organizing protein, Hsp60 (Orru et al. 2003), and α-ketoglutarate dehydrogenase (Cooper et al. 1997).

Functions of mitochondrial TG2 as a PDI

TG2 plays an important role in mitochondrial physiology through its protein disulfide isomerase activity during normal circumstances. PDI activity of TG2 was first demonstrated in vitro using RNase A as a substrate; however, the activity was only about 5% of that of a commercial bovine liver PDI (Hasegawa et al. 2003). There are several proteins that are not members of the PDI-family, but display some degree of PDI activity, including integrins, a spliceosome protein, thyroglobulin, fibronectin, and the elongation factor EF-Tu (Turano et al. 2002). Until recently, the relevance of PDI activity to TG2 function had not been appreciated due to the lack of evidence that physiological substrates of the PDI exist. This raised the question as to whether the PDI activity of TG2 was purely an in vitro phenomenon.

In general, disulfide formation is an enzymatically catalyzed process that occurs in the ER and mitochondrial intermembrane space (Riemer et al. 2009). The formation of disulfide bonds has been thought to be counteracted by numerous reducing enzymes in the cytoplasm, where reduced glutathione is maintained at a very high concentration (~10 mM) (Ostergaard et al. 2004). In mitochondria, TG2 acting as a PDI contributes to the correct assembly of respiratory chain complexes (Mastroberardino et al. 2006; Battaglia et al. 2007). ATP synthase, NADH-ubiquinone oxidoreductase, succinate-ubiquinone oxidoreductase, and cytochrome *c* oxidase were identified as the PDI substrates (Mastroberardino et al. 2006). In line with the observation of the PDI activity of TG2 in mitochondria, several reports documented abnormal phenotypes of TG2 knockout mice associated with altered mitochondrial physiology. In TG2−/− hearts, a defect in ATP was identified as a result of impaired mitochondrial production (Szondy et al. 2006). TG2−/− mice displayed a decrease in motor behavior, which might be due to a defect in mitochondrial energy production (Szondy et al. 2006; Malorni et al. 2008). The defect in ATP generation might be the cause of glucose intolerance and hyperglycemia in TG2−/− animals (Bernassola et al. 2002). Recently, a physical interaction was demonstrated between TG2 and adenine nucleotide translocator 1 (ANT1) in mitochondria (Malorni et al. 2009). Mitochondria isolated from hearts of TG2−/− mice exhibited increased polymerization of ANT1, suggesting that the PDI activity of TG2 regulates the ADP/ATP transporter function (Malorni et al. 2009). ANT1 is a core component of the permeability transition pore complex (PTPC) (Zhivotovsky et al. 2009). Thus, TG2 PDI activity plays an important role in regulating mitochondrial proteins under normal physiological conditions.

Additional intracellular organelles

In rat brain, relatively high TGase activity is present on the external surface of synaptosomes (Gilad and Varon 1985). Involvement of TG2 in neurite formation has been suggested since cellular transglutaminase-specific activity increased 2.5-fold from day 3 to adulthood during mouse brain maturation (Maccioni and Seeds 1986). Involvement of TG2 in neurite formation is further supported by neuronal differentiation promoted by overexpression of TG2 in human neuroblastoma SH-SY5Y cells with selective localization of TG2 to the tips of the neurites (Tucholski et al. 2001).

Although there is little evidence that TG2 is localized to the ER, two putative substrates of TG2, BIP and HSP90B1, are heat shock proteins primarily located in the ER (Orru et al. 2003). Further verification is necessary to confirm if these candidates are genuine substrates of TG2.

Extracellular space

Although it has been initially studied as an intracellular enzyme, there is now little doubt that TG2 is secreted into the extracellular space or onto the cell surface (Verderio et al. 1998; Gaudry et al. 1999a, b). Cell-surface TG2 is proposed to be an important tissue-stabilizing enzyme that is active during wound healing (Haroon et al. 1999). Recently, the functional importance of TG2 as a matrix stabilizer through its protein cross-linking activity, and as an important cell adhesion protein involved in cell survival, was thoroughly reviewed (Collighan and Griffin 2009). Since extracellular TG2 has been described in depth elsewhere (Telci and Griffin 2006; Zemskov et al. 2006;

Collighan and Griffin 2009), molecular interactions of extracellular TG2 and its externalization will be briefly covered here.

Molecular interactions of extracellular TG2 as an adaptor protein

In various cell lines, cell surface TG2 has been shown to interact with α4-, α5-, β1-, and/or β3-integrins (Gaudry et al. 1999a; Isobe et al. 1999; Akimov et al. 2000; Takahashi et al. 2000; Akimov and Belkin 2001a, b). Cell surface TG2 serves as an integrin-binding adhesion coreceptor for fibronectin, thereby promoting cell adhesion and spreading (Akimov et al. 2000; Akimov and Belkin 2001a, b). Recently, it was demonstrated that fibronectin-bound TG2 was directly associated with syndecan-4 in fibroblasts, suggesting its role in tissue repair under situations of matrix breakdown where Arg–Gly–Asp (RGD)-dependent cell adhesion is compromised (Telci et al. 2008). During apoptosis, extracellular TG2 in a guanine nucleotide-bound form was proposed to stabilize efficient phagocytic portals in apoptotic cells via its interaction with MFG-E8, a protein known to bridge integrin-β3 (Toth et al. 2009).

TG2 knockout mice displayed an increase in tumor progression and a reduction in survival rate (Jones et al. 2006). Intratumor injection of TG2 into mice bearing CT26 colon carcinoma tumors resulted in a reduction of tumor growth, likely through modulation of ECM components (Jones et al. 2006). It is not well understood how TG2 suppresses tumor growth. Recently, the C terminus of TG2 was shown to interact with the N terminus of GPR56, a member of a newly described family of G protein-coupled receptors (Xu et al. 2006). GPR56 was shown to play an important role in suppressing tumor growth and metastasis (Xu et al. 2006), which might be related to its role in cell adhesion (Xu and Hynes 2007). Thus, it is tempting to speculate that the extracellular TG2–GPR56 interaction suppresses tumor growth and metastasis (Xu and Hynes 2007).

Externalization of TG2

Despite recent data suggesting extracellular TG2, TG2 externalization into the ECM is far from clear. Externalization appears to depend on an intact fibronectin-binding site in the amino-terminal sandwich region of TG2 (Jeong et al. 1995; Gaudry et al. 1999b). Cells overexpressing C277S TG2 did not deposit the enzyme into the ECM or secrete it into the cell culture medium, implying that its tertiary conformation is critical for its externalization mechanism (Balklava et al. 2002). TG2 externalization from chondrocytes required interaction between FXIIIA and α1β1 integrin (Johnson et al. 2008). Antibody cross-linking of α1β1 integrin mobilized TG2 from chondrocytes, whereas an α1β1 integrin-blocking antibody inhibited TG2 mobilization to the cell surface induced by FXIIIA (Johnson et al. 2008). Although a non-Golgi/endoplasmic reticulum route has been suggested for TG2 externalization, further investigation is necessary to reveal the underlying mechanisms. Once externalized, TG2 is known to be internalized and degraded in lysosomes, which requires LRP1, a member of the low-density lipoprotein (LDL) receptor superfamily (Zemskov et al. 2007).

TGase activity in the extracellular space

Due to low-GTP and high-calcium concentrations in the extracellular space, it is conceivable that extracellular TG2 is an active TGase. However, it may not be the case, because extracellular compartments are maintained at stable oxidizing potentials (Go and Jones 2008). In the presence of standard cell culture medium, such as DMEM, TG2 exogenously added to a culture required dithiothreitol (DTT) for TGase activation (Verderio et al. 2003). Recently, it was proposed that most extracellular TG2 is maintained in the closed conformation, specifically the inactive TGase conformation, despite relatively high extracellular calcium concentrations (Pinkas et al. 2007). In CH-8 cells, a GTP-binding TG2 mutant (K173L) was not efficiently secreted into conditioned media, implying that externalized TG2 is likely in a GTP-bound form (Johnson and Terkeltaub 2005). Moreover, the interaction of TG2 with the extracellular domains of integrin subunits does not require cross-linking activity and facilitates adhesion, spreading, and motility of cells (Isobe et al. 1999; Akimov and Belkin 2001a, b; Stephens et al. 2004). Thus, the secreted TG2 that functions as an adaptor protein is an inactive TGase.

Meanwhile, data suggests that the TGase activity is involved in remodeling of the ECM under normal and pathological circumstances. A selective inhibitor of the TGase, KCC009 (Choi et al. 2005), interfered with remodeling of fibronectin in glioblastomas, both in vitro and in vivo (Yuan et al. 2007). In opossum kidney proximal tubular epithelial cell cultures, high glucose (36 mM) treatment increased extracellular TGase activity, resulting in ECM cross-linking (Skill et al. 2004). TGase-treated collagen substrates were shown to be resistant to protease degradation and facilitated differentiation of human osteoblasts (Chau et al. 2005). Therefore, functions of extracellular TG2 are mediated by its TGase activity, as well as molecular interactions as an adaptor protein. In addition, relatively high level of extracellular TGase activity has been reported under different pathological conditions, such as fibrosis (Griffin et al. 1979). The involvement of extracellular TG2 in pathological conditions has been

described in detail elsewhere (Verderio et al. 2005; Telci and Griffin 2006; Ientile et al. 2007; Siegel and Khosla 2007; Collighan and Griffin 2009). In particular, the TGase inhibition using TG2 inhibitors or TG2 knockout mice slowed the progression of chronic kidney fibrosis and diabetic nephropathy (Johnson et al. 2007; Shweke et al. 2008; Fisher et al. 2009; Huang et al. 2009), suggesting that TG2 is an important therapeutic target for sclerosing kidney diseases.

Although the role of TG2 in disease is not a primary focus of this review, it is clearly worthwhile to note that TG2, in intracellular and/or extracellular compartments, has been implicated in the pathogenesis of a wide variety of diseases: inflammatory diseases including celiac sprue (Molberg et al. 1998; Kim 2006); neurodegenerative disorders including Huntington's, Alzheimer's, and Parkinson's diseases (Lesort et al. 2000a; Ruan and Johnson 2007; Wang et al. 2008); and cancer (Kotsakis and Griffin 2007).

Conclusions and perspectives

In general, TG2 TGase activity seems to become the dominant function of TG2 during apoptotic cell death accompanied by an elevation of intracellular calcium. In addition to TGase activity, at normal intracellular calcium concentrations, the diverse cellular functions of TG2 appear to be attributed to G_h, PDI, and kinase activities. These various biochemical activities of TG2 are differentially regulated depending on its subcellular localization. The microenvironments of the various organelles in which TG2 is found, create unique molecular interactions between TG2 and other proteins. Information on the subcellular locations of TG2, combined with data on the biochemical status of TG2, provides useful clues and insights about the functions and regulation of TG2.

In many other protein localization studies, genetically encoded fluorescent proteins have transformed the research field by allowing for tracing of dynamic protein movements in response to environmental stimuli or internal signaling pathways (Davis 2004; O'Rourke et al. 2005). Knowledge of TG2 subcellular localization has expanded greatly recently, yet studies in the field have rarely utilized fluorescent proteins, rather they primarily rely on biochemical analysis of fractionated samples and/or immunohistochemistry of fixed samples. The application of fluorescent proteins, which would reveal dynamic TG2 translocation in live cells, is anticipated for future studies of TG2.

Acknowledgments This work was supported in part by the National Research Foundation of Korea through the Basic Research Program (2008-05943), the System Bio-dynamics Research Center (2009-0091504), and the Regional Research Universities Program/Medical & Bio-Materials Research Center.

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