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Transglutaminase 2: A Molecular Swiss Army Knife

Soner Gundemir[b], **Gozde Colak**[b], **Janusz Tucholski**[c], and **Gail V. W. Johnson**[a,b,*]

[a]Department of Anesthesiology, University of Rochester, 601 Elmwood Ave., Rochester, NY, 14642, USA

[b]Department of Pharmacology, University of Rochester, 601 Elmwood Ave., Rochester, NY, 14642, USA

[c]Department of Psychiatry, University of Alabama at Birmingham, Birmingham, AL 35294, USA.

Abstract

Transglutaminase 2 (TG2) is the most widely distributed member of the transglutaminase family with almost all cell types in the body expressing TG2 to varying extents. In addition to being widely expressed, TG2 is an extremely versatile protein exhibiting transamidating, protein disulphide isomerase and guanine and adenine nucleotide binding and hydrolyzing activities. TG2 can also act as a protein scaffold or linker. This unique protein also undergoes extreme conformational changes and exhibits localization diversity. Being mainly a cytosolic protein; it is also found in the nucleus, associated with the cell membrane (inner and outer side) and with the mitochondria, and also in the extracellular matrix. These different activities, conformations and localization need to be carefully considered while assessing the role of TG2 in physiological and pathological processes. For example, it is becoming evident that the role of TG2 in cell death processes is dependent upon the cell type, stimuli, subcellular localization and conformational state of the protein. In this review we discuss in depth the conformational and functional diversity of TG2 in the context of its role in numerous cellular processes. In particular, we have highlighted how differential localization, conformation and activities of TG2 may distinctly mediate cell death processes.

Keywords

transglutaminase 2; transcription regulation; transamidase; calcium; GTP; cell death

1. Introduction

Transglutaminase 2 (TG2, EC 2.3.2.13) is a member of the transglutaminase family that catalyzes thiol- and calcium-dependent transamidation reactions. The transamidation reaction catalyzed by the transglutaminase family results in the formation of a covalent bond between the γ-carboxamide group of a peptide bound glutamine residue and a primary amine group. The ε-amino group of a peptide bound lysine can act as the amine donor for the reaction; in this case a covalent crosslink is established between the γ-carboxamide group of the glutamine and the ε-amino group of lysine [1]. TG2 is the most ubiquitously expressed

*Corresponding author: Tel: +1 585 276 3740; fax: +1 585 276 2418. gail_johnsonvoll@urmc.rochester.edu (G.V.W. Johnson).

and most studied member of the family. In addition to its transamidating activity, it has been shown to bind and hydrolyze GTP and ATP. Although ATP/ADP binding has no effect on transamidating activity [2], calcium and GTP/GDP binding inversely regulate the transamidating activity of TG2; that is, TG2 is only active as a transglutaminase when bound to calcium and inactive when bound to GTP/GDP [3, 4]. However, in the GTP/GDP bound form, TG2 is proposed to be involved in regulating signal transduction by acting as a G-protein which transduces a signals from α1-adrenergic, oxytocin and thromboxane receptors [5-7]. Moreover, recent developments show it truly is a multifunctional protein as it is proposed to function as a protein disulfide isomerase (PDI) [8, 9], protein kinase [10, 11], protein scaffold [12, 13]; even as a DNA hydrolase [14].

This unusual multifunctionality may be surprising at first, but a more careful analysis of the evolutionary roots of TG2 might, in part, shed light on the issue. The transglutaminase family has 9 members in higher vertebrates: TG1-7, band 4.2 and factor XIII [1]. All, except band 4.2, retain the ability to catalyze transamidation reactions. Band 4.2 on the other hand, although not catalytically active, still retains important functions such as scaffolding [15]. TG2 is the most widely expressed member of the family and almost all cell types in the body express TG2 to varying extents; it therefore was previously called “tissue transglutaminase”. Furthermore, almost all cell types in the body express at least one additional catalytically active transglutaminase in addition to TG2 [16]. This is a suitable evolutionary background for a protein to develop alternative functions other than the primary function. The primary function of TG2, which is transamidation, being partly carried out by other transglutaminases may have provided a permissive environment for TG2 to develop alternative functions. According to the prevailing theory, all the transglutaminase family members are derived from a single transglutaminase gene; and the first transglutaminase gene shares a common ancestor with calpain-type cysteine proteases [17, 18]. The fact that organisms as primitive as bacteria have the transglutaminase enzyme indicates that the appearance of the first transglutaminase gene occurred very early in evolution [19] (although it is believed that vertebrate transglutaminases did not evolve from bacterial transglutaminase, but rather the evolved convergently). Up through lower vertebrates, only a single transglutaminase gene is found in the genome [17]. This suggests that the gene duplication, which gave rise to two broad classes of transglutaminases, occurred in early vertebrate evolution. As a result of this early duplication, class I (factor XIII & TG1) and class II (TG2-7 & band 4.2) transglutaminases evolved [17]. It is well documented in the literature that gene duplication can result in the acquisition of new functions by the proteins whose genes have been duplicated [20, 21]. This evolutionary background and the functional redundancy offered by other transglutaminases likely lay at the basis of the multifunctionality of TG2. Despite its new functions, TG2 retained its original function of transamidation. However, to what extent transamidation activity of TG2 plays a role in normal physiology is still under discussion.

2. TG2: Transamidating/deaminating function

The first demonstration of transamidation activity was in 1957 in a research article titled “An enzymatically catalyzed incorporation of amines into proteins” [22]. They named the enzyme responsible for this activity “transglutaminase”. By the mid-1980s it was clear that this “transglutaminase” activity was the product of more than one enzyme [23]; a finding that ultimately resulted in the characterization of a whole enzyme family. TG2 has been the most studied member of the family; therefore much of our knowledge about transglutaminase reactions comes from the studies of TG2, and to some extent, from factor XIII as well. Today we know the mechanism of the reaction catalyzed by TG2, which takes place in two steps, in great detail. At the first step, the sulfur of the active site cysteine (C277) performs a nucleophilic attack on the γ-carbon of the peptide bound glutamine side

chain (which is the acyl-donor) and forms a thioester bond between C277 and the substrate. An ammonia molecule is released at this step as a byproduct. At the second step, the thioester bond is attacked by a primary amine or water (which is the acyl-acceptor). If the attacking group is a primary amine (either a small biological amine or the ε-group of a peptide bound lysine), the reaction is called *transamidation*; if it is a water molecule it is called *deamination* [24] (Fig 1).

Transamidation reactions have two major outcomes: addition of the small biological amine to the protein when the attacking group is a small primary amine or formation of an isopeptide bond between acyl-acceptor and acyl-donor peptides (Fig 1b). The addition of a small biological amine can be considered as a post-translational modification and it changes the properties of the substrate proteins. Isopeptide bonds (Fig 1c) are resistant to chemical and physical disruptive forces; therefore they have been suggested to have functional significance in stabilizing the extracellular matrix and in preventing the release of intracellular contents of apoptotic cells to the extracellular milieu [1, 25, 26]. Isopeptide bonds are not the only way to crosslink two proteins: a small amine can mediate the formation of an indirect crosslink (Fig 1d).

The deamination reaction has only one outcome: the conversion of the acyl-donor glutamine residue to a glutamate residue (Fig 1a). For many years, it was believed that the deamination reaction took place only when the availability of primary amines as acyl-acceptor was limiting and water played this role due to its abundance. At best, deamination reactions were believed to be favored only under certain conditions such as lower than physiological pH [27]. It was rarely, if at all, considered that TG2 might actually deaminate a protein even when conditions were permissive for transamidation until a study showed that a glutamine residue in Hsp20 was specifically deaminated while other glutamine residues were transamidated [28]. Further studies showed that specific deamination is observed in other proteins as well [29], and the number of reports examining the structural features of substrates that favor deamination over transamidation is growing [29, 30].

The ‘TRANSDAB wiki database’ (http://genomics.dote.hu/wiki/index.php/Main_Page) currently lists 140 substrates of the transamidase function of TG2 (For more detailed information about TG2 substrates, see Griffin et. al. [1]). It is likely that most of these substrates are modified by TG2 in specific conditions and that these conditions are likely determined by various factors. The factors controlling transamidation/deamination activity and the significance of these activities in cell physiology will be discussed in detail below.

3. TG2: GTPase/G-protein function

The first demonstration that TG2 can bind to and hydrolyze GTP came in 1987 [31]. In that study, the authors also showed that calcium binding and GTP binding were competitive and a transamidating inactive mutant of the active site cysteine retained its GTP hydrolyzing activity. The real breakthrough in the field, however, was the demonstration that TG2 and $G\alpha_h$ are the same protein [6]. In the original study, TG2 was shown to be the functional α-subunit of a G-protein associated with α_1 adrenergic receptor signaling, carrying the signal to phospholipase C-δ1. Further studies from other groups have added to the receptor types with which TG2 can interact. TPα thromboxane A2 receptor [7] and oxytocin receptor [5] were also shown to exploit TG2 as a G-protein. The physiological significance of TG2 acting as a G-protein is still unclear since the number of studies on this topic has been limited and the results are controversial [32, 33].

The inhibitory effect of guanine nucleotide binding by TG2 on its transamidase activity has been very well documented [31]. The approximations for IC_{50} of GTP range from ~300 nM to 6 μM [34, 35]. Determining the affinity of TG2 for calcium ions has been more difficult

since there are at least 3 (more likely 6) calcium binding sites with very different affinities for calcium. However what is very clear is that the intracellular average GTP/calcium ratio (~150 μM / ~100 nM) is more than sufficient to keep TG2 in a relatively latent state as a transamidase. Certain splice variants or single site mutants of TG2 that lack guanine nucleotide binding capability are more prone to exhibit transamidating activity *in situ* as expected [3, 34, 36, 37]. Furthermore, groups that have studied GTP-binding defective forms of TG2 have concluded that the loss of GTP binding renders the enzyme more proapoptotic [36, 38, 39]. Nonetheless, it is still controversial whether the loss of guanine nucleotide binding makes TG2 more proapoptotic *per se*, or is it because the loss of binding promotes the transamidating activity of the protein [36, 38]. Although further studies are required to clarify this issue, it should be noted that these are not mutually exclusive hypotheses and both could be true depending on the context.

In addition to cell death, different isoforms may affect cell differentiation in opposing directions. In a recent study [39], it was found that GTP-binding defective short form of TG2 (TG2-S) induced neuroblastoma cell differentiation, whereas long form (TG2-L) suppressed it. Importantly, transglutaminase inhibitors prevented TG2-S induced differentiation, which strongly suggests that the main driver of differentiation is the elevated transamidating activity due to GTP binding [39]. This conclusion is also in line with a previous report from our group [40].

4. TG2: Kinase function

Although the GTPase activity of TG2 was associated with its suggested G-protein function, no functional outcome of the ATPase activity was proposed until Mishra *et. al.* provided data suggesting that TG2 has intrinsic kinase activity [10]. In their original paper [10] and in the follow up studies that came from the same laboratory [11, 41-43], the authors reported that TG2 phosphorylates insulin-like growth factor-binding protein-3 (IGFBP-3) [10], histones [11], p53 [42] and retinoblastoma protein [43]. The in vitro phosphorylation experiments were conducted with a variety of TG2 preparations from commercial to recombinant TG2, all of which exhibited kinase activity. The use of different TG2 preparations decreases the chance of the presence of a contamination and clearly indicates that in vitro TG2 can act as a kinase. However, whether TG2 does or does not act as a kinase *in vivo*; and if it does, how significant is this activity in normal physiology, is still an open question. Furthermore, the authors also provide evidence that another member of the transglutaminase family, factor XIII, has kinase activity as well [42]. For this to be true, factor XIII must exhibit ATPase activity. This issue has not been studied, but an early report showed that factor XIII can be inhibited by ATP [44], which is an indication of ATP binding to factor XIII and supportive of the claim that factor XIII has kinase activity.

5. TG2 as protein disulphide isomerase (PDI) and a regulator of mitochondrial function

Compared to the proposed kinase activity, the PDI activity of TG2 is relatively well-established. First of all, there are several studies describing proteins with both transamidase and PDI activities [45-47]. Therefore, the demonstration of the PDI activity of TG2 was not without precedent. The original report which showed TG2 has PDI activity [8] clearly demonstrated that transamidase active site cysteine (C277) is not required for PDI activity. In this study TG2 converted inactive/denatured RNase A into an active enzyme by the formation of correct disulphide bonds. PDI activity of TG2 was shown to be independent of its transamidation activity, did not require calcium and was not inhibited by nucleotides. Given that concentrations of nucleotides are high and calcium are low in the cytosol, the authors suggest that TG2 may be acting as a PDI in this compartment [8]. Similar to the

kinase case, factor XIII was shown to have PDI activity as well [48], independent of its transamidating activity.

The first evidence showing that TG2 acts as a PDI *in vivo* came from the studies focusing on mitochondrial TG2 [9]. In this study, the authors found a decrease in the disulphide bond content of mitochondrial respiration complexes in tissues from $TG2^{-/-}$ mice; suggesting that TG2 acting as a PDI facilitated the formation of disulfide bridges in mitochondrial complex I, II and V [9]. They also found a decrease in ATP levels in the $TG2^{-/-}$ mice cardiomyocytes and skeletal muscle cells, and suggested a causal relationship between the absence of TG2, decreased disulphide bond content and decreased ATP levels [9, 49]. Although this causality is yet to be established, the existence of this correlation is an important observation itself. In a recent study, a mitochondrial protein, the adenine nucleotide translocator 1 (ANT1), was shown to interact with TG2 and suggested to be a substrate for both transamidase and disulphide isomerase activities of TG2 [50]. ANT1 is an important permeability transition pore (PTP) subunit, therefore it is very important for mitochondrial function. Data was presented suggesting that ANT1 polymerization is prevented by TG2's PDI activity. [50]. In $TG2^{-/-}$ mice ANT1 was found to be a more polymerized state than in wild type mice, and ADP/ATP transporter activity was enhanced in heart mitochondria from $TG2^{-/-}$ mice. Acceleration of ANT1 transporter activity might exert protective effects in the case of an impairment of mitochondrial activity. Interestingly, TG2-/- MEFs are particularly sensitive to atractyloside which binds to outward facing conformation of ANT1 and blocks it suggesting that TG2 affects the orientation/conformation of ANT1. Considering that PDI activity of TG2 might also contribute to the stabilization of the respiratory complexes [9], the overall ATP homeostasis could be affected by the absence of TG2, or with upregulation of TG2 due to stressors [50].

Although many studies suggest that the PDI activity of TG2 is crucial for its role in mitochondrial function, it is not always clear how TG2 modulates mitochondria-dependent processes. In an earlier study, TG2 overexpression led to increased cell death in response to staurosporine (STS) treatment possibly through increased cytochrome c release [51]. In this study, TG2 overexpressing cells showed hyperpolarized mitochondria and elevated levels of reactive oxygen species (ROS) under normal conditions. Inhibition of mitochondrial transition pore opening with Cyclosporin A (CsA) or adding antioxidants like N-acetylcysteine (a thiol supplier) or L-acethylcarnitine (natural compound to increase energy charge) prevented STS-induced cell death in TG2 overexpressor cells, however there was no affect of CsA on mitochondrial hyperpolarization due to TG2 overexpression. Therefore, the authors suggested that TG2 may alter the redox state of the cell which could contribute to the potentiation of STS-induced cell death [51]. In contrast, overexpression of TG2 in striatal cells enhanced mitochondrial membrane depolarization in response to thapsigargin, although it did enhance thapsigargin-induced ROS production as well as increase cytochrome c release, caspase-3 activation and cell death [52]. Given these conflicting results, it is likely that the potentiation of cell death with increased TG2 expression is not directly due to mitochondrial hyperpolarization. It has also been suggested that a putative BH3 domain in TG2 may be involved in the increase in STS-induced cell death in TG2 overexpressing cells [53]. In this study an eight amino acid region (204-212) in the catalytic domain of TG2 that showed 70% identity with the BH3 domain of Bcl-2 [53]. It was suggested that this region could result in dimerization of TG2 taking on a conformation like Bax. Deletion of the putative BH3 domain or mutation of L204E (the amino acid critical for homo/hetero-dimerization of Bax) abolished the sensitization of TG2 overexpressing cells to STS-induced cell death. However, the authors did not address the fact that deleting the putative BH3 domain of TG2 might also disrupt the transamidation activity of TG2. Therefore, the prevention of cell death sensitization could be due to absence of transamidation activity in the mutant forms. The same study also showed that TG2 can

interact with Bax, and the BH3 domain of TG2 causes translocation of Bax to mitochondria and induces cytochrome c release [53]. However there was no interaction between TG2 and Bcl-2 or Bcl-X_L, the antiapoptotic proteins. It is also suggested that TG2 transamidates Bax protein, leading to its polymerization at the mitochondria level. In the data, the interaction between TG2 and Bax was increased in response to cell death induction with STS. However, this result might be also due to the fact that cell death induction can cause an increase in the expression level of Bax protein, therefore the amount of protein that was pulled down due to the interaction is higher [53].

A significant issue that needs to be considered when evaluating the effects of TG2 on mitochondrial function is the fact that findings indicate that TG2 is not inside mitochondria (Fig 2). Although there is calcium-dependent transamidating activity in purified mouse mitochondria and mitoplast, the activity was similar in mitochondria from wild type and TG2-/- mice. In addition, TG2 could not be detected in the mitochondrial fractions [54]. However, it does not exclude the possibility that TG2 might interact with or modify the proteins present on the outside of the mitochondria as has been suggested [53]. Indeed the majority of TG2 is loosely associated with mitochondria as it is easily removed by digitonin treatment [50] (Fig 2). The possibility of other transglutaminases being in the mitochondria, as well as the lack of a good antibody which recognizes mouse TG2 specifically, have hindered studies on the role of TG2 in the mitochondria. Further studies are clearly needed to fully delineate the role of TG2 in modulating mitochondrial function.

6. Structure/function relationship of TG2

The multifunctionality of TG2 is dependent on the structural features of TG2. TG2 is comprised of 4 domains: an N-terminal β-sandwich domain (aa 1-140), the catalytic core (aa 141-460) and two C-terminal β-barrel domains (aa 461-586 & 587-687) [55]. The crystal structure of TG2 is available in its GDP bound closed form [55], ATP bound closed form [56] and inhibitor bound open form [30], which have been invaluable for our understanding of the structure and function of TG2.

The site of transamidating activity is composed of the catalytic triad of cysteine proteases (Fig 3); cysteine 277 (C277), histidine 335 (H335) and aspartate 358 (D358), which are the critical residues for transamidating activity [55]. The cysteine to serine mutation at the position 277 (C277S) has been extensively used to inactivate the transamidation function of TG2 [1]. Although this mutation knocks out all transamidating activity, it results in a conformational change which greatly impairs the GTP/GDP binding capability as well and increases the propensity of the protein to exist in an open conformation [3, 36]. In addition to the catalytic triad, two conserved tryptophan residues (W241 [Fig 3] and W332), located at the opposite sides of the “catalytic tunnel”, are critical for the transamidating activity, since these residues stabilize the enzyme-thiol intermediate that forms during catalysis [30, 57]. Mutating W241 to an alanine (W241A) knocks out transamidating activity without any effect on GTP/GDP binding [30, 36]. In contrast, a W332F-TG2 mutant was significantly impaired in its ability to bind GTP/GDP [57]. A threonine residue (T360) at the entrance of the catalytic tunnel controls the entry of the acyl-acceptors for the second step of the catalysis. The mutation of this residue (T360A) very significantly increased the preference for deamination over transamidation [30]. Another important residue in the catalytic site is the tyrosine residue at the position 516 (Y516). As shown in Fig 3, a hydrogen bond forms between C277 and Y516 in the closed conformation of TG2 which is believed to further stabilize the closed conformation and keep the enzyme inactive [30]. Although the exact effect of loosening this hydrogen bond is not clear, mutating this residue to phenylalanine (Y516F) rendered TG2 more prone to an open conformation [3].

ATP and GDP were found to bind the same nucleotide binding pocket [55, 56]. It is a perfect example of a tertiary pocket, with contributing residues from the catalytic domain, β-barrel-1 and β-barrel-2 domains (Fig 4). It is composed of at least ten residues, many of which are involved both in adenine and guanine nucleotide binding; however, S482 and R580 were found to be involved only in guanine, not adenine, nucleotide binding [56]. Therefore, if one wants to decrease the affinity of TG2 for guanine nucleotides selectively, the arginine residue located at position 580 (R580) is the best candidate. It interacts with the guanine nucleotide at several points and its mutation to alanine (R580A) resulted in the almost complete loss of GTP/GDP binding activity and a disinhibition of transamidase activity [3, 36, 58]. It should be noted, however, that the R580A mutation does not necessarily decrease the GTPase activity of TG2. TG2, due to its high affinity for GDP as well as GTP, is likely to spend significant time in the GDP bound form after the hydrolysis of GTP. Therefore, there is a prolonged 'docking time' for the replacement of GDP with a new GTP molecule. The R580A mutation decreases the affinity for both GTP and GDP; therefore, it has opposing effects on the GTP hydrolysis rate. On the one hand, this mutation enhances the rate by decreasing the affinity for GDP – hence less docking time is required; on the other hand it reduces the rate by decreasing the affinity to GTP. In addition, this mutation might have an effect on the catalysis process *per se*. Overall, the effect of this mutation on GTP hydrolytic rate is to be determined, but it is clear that the R580A mutant spends much less time in guanine nucleotide bound form compared to wild type. Lastly, although not shown in Fig 4, S171 and K173 residues are very important for GTPase function [38, 59].

Unlike the nucleotide binding pocket, we do not have structural information about the calcium binding motifs since the crystal structures were obtained in the absence of calcium [30, 55]. However, the structures of two transglutaminases are known in the calcium bound state: factor XIII [60] and TG3 [61, 62]. Structural prediction studies based on other transglutaminases, calorimetric studies and site directed mutagenesis studies have been conducted to help elucidate the calcium binding sites of TG2 [63-65]. The prevailing consensus is that TG2 has 6 calcium binding sites all of which are located in the catalytic domain [65]. There is little enthalpy change due to calcium binding, which suggests the binding energy is used for entropic change, which means structural change. It is plausible to argue that the free energy required for the dramatic conformational change comes from calcium binding, at least in part. Furthermore, there is evidence that suggests there is cooperativity among binding sites [65], that is, binding of calcium to certain sites might trigger the binding of additional calcium ions. The highest affinity site is ^{228}VNCNDDQGV^{236}, and mutating this site prevents binding of more than one calcium ion due to cooperativity. Mutating any of the calcium binding sites decreased transamidating activity, but mutating 3 of the 6 binding sites completely abolished it. As expected, mutating calcium binding sites does not have a dramatic effect on GTPase activity [65]. Overall, although we lack the crystallographic evidence for calcium binding, studies exploiting alternative methods have partially made up for this shortcoming.

Theoretically and experimentally, there is little doubt that transamidase activity of TG2 tends to increase as the [calcium] / [guanine nucleotide] ratio increases. Therefore, it is reasonable to expect TG2 to be largely silent as a transamidating enzyme inside the cell where this ratio is very low; and enhanced activity outside the cell where there is virtually no GTP or GDP, but millimolar levels of calcium. However, experimental evidence suggests otherwise: extracellular TG2 appears to inactive unless there is a physical or a chemical stressor [66]. This observation argues for the existence of additional control mechanisms for transamidating activity of TG2 other than calcium and guanine nucleotides. Redox state of the environment might be important for the regulation of TG2 activity as this protein is suggested to bear at least five intramolecular disulphide bridges [8]. In a recent study, two of

these disulphide bonds (between C370-C371 and between C370-C230) were shown to play role in inactivation the transamidase activity of TG2. A disulphide bond that forms between C230 and C370 facilitates the formation of a disulphide bond between C370 and C371 which results in inactivation of the transamidase activity of TG2. [67]. The formation of the C370-C230 disulphide bond occurs in a less oxidizing environment than the C370-C371 bond, and thus promotes inactivation of TG2 even in the presence of calcium. The authors argue that the presence of these disulphide bonds in high calcium environments, like extracellular milieu, helps to keep TG2 inactive as a transamidase. Intriguingly, one of these inactivating disulphide bridges, C230-C370 bond, is observed in the ATP bound closed form [56]. Given that ATP has been reported not to inhibit TG2 [2], or was 100 times less effective in inhibiting the enzyme compared to GTP [68], it maybe that ATP-bound TG2 is amendable to the calcium-induced open conformation – and possibly – subsequent inactivation in an oxidizing environment by formation of the C370-C230 disulphide bond.

It is not uncommon to see complex regulatory mechanisms, such as redox control, modulate different activities of multifunctional proteins like TG2. It would be expected to see very high utilization of posttranslational modifications to regulate TG2, as well; but, there is only a limited number or studies reporting posttranslational modifications of TG2. However, this issue is beginning to be addressed as two recent publications provide evidence that TG2 is SUMOylated [69] and S-nitrosylated [70]. In the first study, the authors found ROS-dependent SUMOylation of TG2, which decreased its ubiquitination and degradation. This shows that the redox state has a more complicated effect than simple inactivation of transamidase activity [69]. In the second study, it was shown that nitric oxide mediates S-nitrosylation of TG2 which inhibits transamidase activity [70]. Given the role of nitric oxide in endothelial cells, this regulatory mechanism may be especially important for vascular TG2. Indeed, the authors in the same study found that the aging process decreases S-nitrosylation of TG2 which in turn may contribute to arterial stiffness in the elderly [70]. Lastly, it has been reported that TG2 is phosphorylated by PKA and that this phosphorylation regulates its binding to 14-3-3 protein [41]. The report solely relies on *in vitro* data, thus awaits confirmation by *ex vivo* and *in vivo* studies. Further studies might reveal additional posttranslational mechanisms that are involved in the modulation of TG2 activity adding more complexity to the already sophisticated regulatory network.

As opposed to the relatively well-studied mechanisms regulating its enzymatic activity, there is very little information regarding what controls the subcellular localization of this protein. TG2 is primarily cytosolic with small quantities found in the nucleus [71, 72], on the outer side of the plasma membrane [73, 74], and secreted to the extracellular matrix [75]. Intriguingly a recent study suggested that microvesicles which are shed by certain cancer cells contain TG2, which is not only a process by which TG2 could be externalized, but could also result in the transfer of TG2 to other cells [76]. Externalization of TG2 by a mechanism that involves targeting to perinuclear recycling endosomes has also been recently described [77]. Recently two putative nuclear localization signals (^{259}DILRR^{263} and ^{597}PKQKRK^{602}) have been identified in TG2, but mutation of one site resulted in inactivation of the transamidating activity and therefore was not used in the study to examine TG2 localization. In addition, mutation of the second site was combined with a nuclear export signal [78] and therefore the effects of these putative nuclear localization signals on the intracellular localization of TG2 remain undetermined. Although these recent studies are intriguing, the mechanisms involved in the localization of TG2 to these different cell compartments still require further examination.

7. Control of TG2 expression

The expression of TG2 is thought to be mainly controlled at the transcription level [1]. The human TG2 promoter is extremely responsive to various activators [79] (Fig 5). Sp1 binding sites are very commonly observed in human promoters and the TG2 promoter has 4 Sp1 binding sites [1, 79, 80]. The most well known activators of TG2 are retinoids and this activation is mediated via a tripartite response element in the human TG2 promoter located approximately 1.7 kb upstream of the transcription start site [81]. The TG2 promoter has a functional transforming growth factor β1 (TGF-β1) response element approximately 0.9 kb upstream of transcription start site [82]. However, the relationship between TG2 and TGF-β is a complicated one, and will be discussed in more detail below in the section on cell surface TG2: A cue for survival, proliferation and migration. Response elements for two very important stress induced transcription factors are located on the TG2 promoter: NF-κB [79, 83] and hypoxia inducible factor (HIF) [84] (Fig 5). Tumor necrosis factor α (TNF-α), which is highly upregulated under inflammatory stress, is another well known inducer of TG2; other inflammatory cytokines, such as interleukin 1 β (IL1β) or interleukin 6 (IL6), can also upregulate TG2 [85-88]. It would not be surprising to see the characterization of new response elements on TG2 promoter.

The TG2 promoter not only serves as a venue for induction, but it also plays a role in silencing of TG2 expression. TG2 expression generally correlates with aggressiveness in cancer cells [89]. In a breast cancer cell model, it was shown that CpG islands in the TG2 promoter are hypermethylated, therefore TG2 expression is epigenetically silenced in less aggressive cell types [90]. Very similar observations were made in non-small cell lung cancer [91] and in glioma [92]. These studies provide strong evidence for the utilization of epigenetic methylation as a means to silence TG2 expression in various cancers. Another mechanism for downregulating TG2 promoter activity involves histone deacetylases (HDACs). Liu *et. al.* showed that in a neuroblastoma model N-myc protein transrepressed the activity of the TG2 promoter, hence TG2 expression, by recruiting HDAC1 to an Sp1 binding site on TG2 promoter [93]. These studies demonstrate how actively the TG2 promoter is regulated by transactivation, transrepression and epigenetics; which may be an indication of the importance of TG2 expression to the cell. Overall, TG2 is generally upregulated under stress conditions and it can be categorized as a stress response protein [94]. This upregulation in TG2 levels is probably part of a cellular protective response. However, if the insult is excessive and the cellular demise is inevitable, TG2 may also facilitate cell death [1].

Very intriguingly, several stress conditions and inducers have been shown to result in the expression of different isoforms of TG2 [34, 95-99]. In response to retinoic acid [95, 96] and cytokines [99] different splice variants were observed in different models. Similarly, central nervous system pathologies such as Alzheimer disease [97] and acute spinal cord injury [98] caused upregulation of certain isoforms. The most important common feature of these variants is that they all lack certain segments of the C-terminal region of TG2 and there are variations in the amino acid sequence at certain points. Several amino acids in the C terminal region contribute to the guanine nucleotide binding pocket; therefore deletion of a these residues significantly diminishes the affinity for GTP [34]. It is largely unknown how these splice variants are generated. The sequence differences make the conventional alternative splicing mechanisms unlikely. At least some of these variants are likely formed due to intronic read-throughs [34, 95, 97-99]. Further studies are required to clarify the mechanisms by which these variants are generated and the role of each variant in normal physiology and pathology.

8. TG2 regulates cell survival/death processes

The multifunctional nature of TG2 complicates determining the role it plays in cell physiology and pathology. For example, the role of TG2 in the cell death/survival process is extremely complex, and circumstances in which TG2 acts as a prodeath or prosurvival protein is an area of active investigation. Relatively early studies from our lab clearly show that TG2 modulates caspase-dependent programmed cell death [100, 101]. However, there is evidence indicating that TG2 can modulate other modes of controlled cell death. TG2 activity was reported to induce the release of apoptosis inducing factor (AIF) from mitochondria in pancreatic ductal adenocarcinoma cells and trigger caspase-independent apoptosis [102]. Very intriguingly, TG2 was found to inhibit autophagy in the same cell line [103]. A recent report from a different group showed that autophagosomes cannot mature in the absence of TG2 and acidification does not occur [104]. Neither of the studies determined whether transamidase activity is required for the role of TG2 in autophagy [103, 104]. A recent paper, however, provided evidence that suggests transamidase activity might be involved in the final steps of autophagosome maturation, therefore is required for autophagy [105]

Many of the initial studies which investigated the relationship between TG2 and cell death relation were conducted in neuronal models. This was mostly because TG2 is the most prevalent neuronal transglutaminase [106-108], and in the human brain increases in TG2 expression have been observed in a number of chronic or acute neuropathological conditions [109]. Due to this upregulation, it was hypothesized that TG2 facilitates neuronal cell death in neurodegenerative diseases. This hypothesis has been supported by studies in which crossing of Huntington disease transgenic mice with TG2-/- mice resulted in a significant delay in the onset of motor dysfunction and prolonged survival of the Huntington disease transgenic mice [110, 111]. However, the reason that TG2 deletion in the Huntington disease mouse model is beneficial is unclear; TG2 knockout mice *per se* are phenotypically normal with no apparent deficits in the major apoptotic pathways [33, 112]. Further analyses are required to determine how deletion of TG2 is protective in these Huntington disease models.

Further evidence for the cell death promoting function of TG2 in neurons has come from studies in transgenic mice that overexpress human TG2 selectively in neurons (hTG2 mice). Even though phenotypically normal, hTG2 mice exhibited a dramatic increase in neuronal damage in the most sensitive hippocampal regions in response to kainic acid treatment [113]. However, there is also evidence supporting an alternative theory: that TG2 upregulation might be the neuron's response to stressful conditions and might be protective. For example, as will be discussed below, TG2 is upregulated in brain ischemia models, but this upregulation is protective, possibly due to nuclear accumulation and regulation of transcription [36, 114, 115]. . Clearly not only the cellular context, but also the specific stressor or pathological conditions are important factors in determining whether TG2 will facilitate or ameliorate cell death processes.

Overall, the mechanisms by which TG2 facilitates cell death or protects cells are complex, case specific and still under investigation. However, there are two relatively well described mechanisms that are widely exploited by TG2 to control cell death/survival processes: *(i)* mediating cell to extracellular matrix (ECM) contacts, thereby triggering cell survival signaling; *(ii)* controlling the activities of transcription factors directly or indirectly to promote survival or facilitate demise.

9. Cell surface TG2: a cue for survival, proliferation and migration

The fact that TG2 is present on the cell surface has been known for a long time, but until recently it was widely believed that the major role of extracellular TG2 was to stabilize ECM through forming isopeptide bonds among ECM proteins [116-118]. Although this function of TG2 is still thought to be important, recent evidence clearly show that its function is more complicated than originally thought. In a matter of several years, a paradigm shift occurred in the field which was triggered by the realization that TG2 is an extremely important cell surface protein with consequential functions for regulating cell survival, proliferation and migration [74, 75].

The ground breaking work was done in 1992 by Gentile *et. al.* [119]. In this initial study, the authors showed that overexpression of TG2 in a fibroblast model increased adhesion of the cells to the substratum [119]. However, this important study fell short of showing that it was extracellular TG2 mediating the increased adhesion. As a matter of fact, the first direct demonstration of cell surface TG2 came seven years later with the evidence demonstrating that the protein is concentrated at the points of cell adhesion [120]. It was long known that TG2 can bind plasma fibronectin with very high affinity [121]. TG2 – ECM fibronectin interaction and the importance of this binding in mediating TG2's role in cell adhesion was shown by the same group [122].

Fibronectin is a principal TG2 interactor in the ECM, but it is definitely not the only one. TG2 can directly interact with at least 3 different β-subunits of the integrin family: β1, β3 and β5 [74, 123]. TG2 association with β1 integrin did not prevent integrin-fibronectin association; they formed ternary complexes [123]. TG2 – integrin – fibronectin ternary complexes are clearly involved in cell adhesion and migration [123]. Importantly, these ternary complexes activate certain intracellular pathways, such as the focal adhesion kinase (FAK) and its downstream PI3K/Akt survival pathway, resulting in increased cell survival and migration [124]. Furthermore, integrin-dependent signaling to RhoA and its downstream target Rho-associated coiled-coil containing serine/threonine protein kinase (ROCK) is increased by cell surface TG2 [73]. In a different study, TG2 – fibronectin complexes supported cell adhesion and the formation of RhoA-dependent focal adhesions, which in turn promoted cell survival [125]. In summary, cell surface TG2 is very important for integrin mediated cell signaling.

One of the consequential findings of cell surface TG2 research was the demonstration that the main role of TG2 in cell adhesion and spreading was not dependent on its transamidase activity. Catalytically inactive forms of TG2 retain their ability to function as ‘integrin binding adhesion coreceptors for fibronectin’ and mediate attachment and migration on fibronectin surfaces [75, 123]. Indeed, it was shown that under resting conditions, cell surface TG2 is not catalytically active, but activated upon injury [66]. These important studies illuminated the importance of TG2 independent of its transamidase, also probably GTPase, activity. There is growing evidence that this ‘scaffolding’ function might be important for intracellular TG2, as well as cell surface TG2 [36].

Another interactor of TG2 in the ECM is TGF-β. As we mentioned above, TGF-β can and does induce TG2 expression [82]. Intriguingly, the extracellular transamidase activity of TG2 was shown to be important for plasmin-mediated activation of TGF-β [126]. TG2 was also shown to increase TGF-β expression through activating the NF-κB pathway [127]; thereby increasing the synthesis and deposition of matrix proteins. This result is in line with the evidence obtained from TG2 knockout mice. Shweke *et. al.* showed that TG2 knockout mice were protected against fibrosis due to decreased rate of collagen I synthesis. This decreased synthesis rate was, the authors conclude, the result of decreased TGF-β activation

in the absence of TG2 [128]. It is also interesting that TG2-/- show significantly less pulmonary fibrosis than wild type mice in response to bleomycin and TGF-β causes an increase in membrane localized TG2 in cell models [129]. From these studies, it appears that there is a positive feedback loop between TG2 and TGF-β.

The interactor list of extracellular TG2 is getting longer as new interacting proteins are being reported almost every year. The TG2 interacting proteins and glycans other than fibronectin and integrins include: Collagen, vitronectin, osteopontin, nidogen, laminin, osteonectin, osteocalcin [1], fibronectin α, fibronectin γ, substance p, endostatin, heparin [130], syndecan-4 [131], amyloid β-peptide [132, 133], MMP-2 [134] and VEGFR-2 [135]. The role of these interactions in cell physiology requires further studies.

It is clear that the presence of TG2 on the cell surface increases attachment to fibronectin surfaces; however the effect of this attachment on migration and spreading is not entirely clear. Both increased and decreased cell migration as a result of TG2-mediated binding to fibronectin or collagen has been reported [13, 75, 136]. This could be explained by the differences in model systems. The ability to migrate is especially important in inflammation and cancer. Metastatic capacity of cancer cells depend on their ability to migrate. Several studies from Mehta and his colleagues have shown that TG2 increases the invasiveness and metastatic capacity of certain cancer cells [89, 137, 138]. It should be noted however, that any increase in metastatic capacity is most likely mediated by the scaffolding function of TG2, and when transamidating activity is involved, TG2 might actually decrease the migration and invasion [139, 140]. Evidence is accumulating showing the role of TG2 on the migration of inflammatory cells. In an elegant study, Mohan *et. al.* identified TG2 as a receptor that mediates T-cell transendothelial migration [141]. In this study, TG2 was on the surface of endothelial cells, simply guiding the migration of T-cells. This is in contrast to a more recent study which proposed that enhanced TG2 expression in migrating cells mediates migration through cell surface TG2 – fibronectin interaction [142]. In this study, the authors speculate that cell surface TG2 contributing to astrocytic infiltration of multiple sclerotic (MS) lesions, thus contributes to inflammation. In a similar study, TG2 immunoreactivity was found in MS lesions and it was postulated that the extracellular TG2 mediates astrocyte adhesion to fibronectin and migration in the extracellular matrix of MS lesions [143]. The role of cell surface TG2 in inflammation is not limited to guiding migration. Evidence from TG2 knockout mice revealed that the phagocytic capacity of TG2 null macrophages to engulf apoptotic cells is impaired [144, 145]. A more detailed analysis revealed that in the absence of TG2, integrin β3 (which is involved in the uptake of apoptotic cells by macrophages and is a TG2 interactor) cannot efficiently recognize the apoptotic cells to be engulfed, among other deficiencies in phagocytic portal formation [146, 147]. These deficiencies lead to an age dependent autoimmunity in TG2 knockout mice due to impaired clearance of apoptotic bodies [144, 148]. Other groups have also provided supporting evidence to the role of TG2 in apoptotic cell clearance by macrophages [149]. In short, the role of TG2 in cell migration may represent a therapeutic intervention point in metastatic cancers and inflammatory diseases.

A very interesting mechanism by which TG2 inhibits cell migration was recently defined that warrants mentioning. TG2 was found to act as a ligand for the atypical G-protein coupled receptor, GPR56 [150, 151]. The binding of TG2 to GPR56 inhibited tumor growth and metastasis. Due to this inhibitory effect, the authors suggest, TG2 might act as a host defense against metastatic cells [151]. Interestingly, downregulation of GPR56 was observed in highly metastatic melanoma cells, and hence there is a bypassing of TG2's anti-metastatic effect [150]. Importantly, it should be noted that extracellular TG2 is mostly associated with increased migration and invasion, and the GPR56-dependent anti-metastatic effect of TG2 in melanoma cells is an exception to this general observation.

It is a well-established notion that the increased attachment to ECM has a positive effect on cell survival; because, in the absence of this attachment, normally adherent cells die through a process called ‘anoikis’ [152]. Cell surface TG2 hence promotes survival by promoting attachment to the matrix, thereby protects cell from anoikis [125]. Therefore, TG2 not only promotes migration and spreading, but also promotes survival.

As an important side note, the TG2 inhibitor KCC009 was reported to ‘block the remodeling of fibronectin in the ECM in glioblastomas in both *in vitro* and *in vivo* studies and sensitize glioblastomas to chemotherapy’ [153]; which prompted the authors to conclude that TG2 is involved in fibronectin remodeling and resistance to chemotherapy. In a more recent study, the same group reported that molecules related to KCC009, but lack the TG2 inhibitory effect, retained their prodeath effects [154]. This observation highlights the importance of caution while interpreting the results obtained by TG2 inhibitors.

The last decade witnessed the exponential growth of publications about cell surface TG2, which has proven to be extremely important in many physiological and pathological processes. In the current decade, it is not unreasonable to expect the translation of this new body of information into pharmaceutical and possible clinical studies.

10. Transcriptional regulation by TG2

Although the major TG2 pool inside the cell is cytosolic, the presence of nuclear TG2 is well-documented [71, 72] (Fig 2). More interestingly, a pattern is emerging about TG2: stress induced nuclear localization. Increasing numbers of stressors are being reported to increase the amount of TG2 in the nucleus. Earlier reports suggested that sphingosine-induced cell death [14], glutamate induced excitotoxicity [155] and maitotoxin induced calcium increases [71] causes nuclear accumulation of TG2. A more recent report, using a different cell model and an ionophore, reinforced previous findings which showed that elevated intracellular calcium levels increase translocation of TG2 to the nucleus [156]. Tatsukawa *et. al*. demonstrate very clearly that the ethanol induced apoptosis in hepatic cells was accompanied by a significant TG2 accumulation in the nucleus [157]. We [114, 115] and others [84] have shown that hypoxic/ischemic stress significantly increases the levels of nuclear TG2. In addition to stress conditions, Balajthy et. al. reported that TG2 translocated into the nucleus during the differentiation of NB4 cells to neutrophil granulocytes [158]. Therefore, it is reasonable to expect a functional outcome from this nuclear accumulation, and it is becoming evident that this outcome is most likely transcriptional regulation [78, 84, 114, 115, 157, 159].

A growing number of studies indicate that TG2 plays a role in modulating transcriptional processes (Fig 6). Our previous studies focus on the role of TG2 in HIF signaling. We have shown [36, 114, 115] that TG2: *i)* accumulates in the nucleus in response to hypoxic and ischemic stress, *ii)* can interact with HIF1β and suppress HIF-dependent transcription (Fig 6), *iii)* is protective in *in vivo* and *ex vivo* ischemia models and, *iv)* nuclear localization of TG2 enhance its protective effect.

In addition to our studies, a very recent study showed that nuclear TG2 may mediate transcriptional dysregulation in cellular models of Huntington disease [78]. This is intriguing given the fact that TG2 is upregulated in Huntington disease patient brains [160] and there is increasing evidence that malfunctioning transcriptional regulation likely contributes to the progression of Huntington disease [161]. In the study by McConoughey and co-workers [78] data was presented suggesting that: *i)* TG2 suppresses PGC-1α and cyt-c expression at the transcriptional level (Fig 6); *ii)* nuclear localization of TG2 is necessary for the transcriptional suppression; *iii)* TG2 is recruited to the promoters of cyt-c and PGC-1α; *iv)* recruitment of TG2 to the cyt-c promoter is increased at least ten times in

mutant huntingtin cells; *v)* wild-type TG2 suppressed the promoter activity of cyt-c, whereas transamidating-inactive TG2 did not, and *vi)* an inhibitor of TG2, ZDON, increased PGC-1α and cyt-c expression [78]. As an important note, all these TG2-mediated effects were observed in the presence of mutant huntingtin and there were little, if any, effect in wild type huntingtin expressing cells [78].

The work done by Tatsukawa *et. al*. constitutes another important example of TG2-mediated transcriptional regulation [157]. In this study, the authors show that: *i)* ethanol induces nuclear accumulation of TG2, *ii)* TG2 crosslinks and inactivates Sp1 in response to ethanol treatment (Fig 6), *iii)* ethanol-induced apoptosis involves TG2 mediated inactivation of Sp1 and *iv)* TG2-dependent apoptosis accompanies reduced expression of Sp1 targets. This detailed study shows that TG2 suppresses transcription of a certain set of genes (a subset of Sp1 target genes) in a transamidating activity dependent manner. The major shortcoming of this study was the lack of the use of transamidating inactive mutants. Although the authors used TG2 inhibitors cystamine and putrescine [157], these are not selective or specific inhibitors.

Another example of TG2 regulating transcriptional processes was in a report by Ahn *et. al*. [159]. In this study they showed that TG2 overexpression suppresses matrix metalloproteinase-9 (MMP-9) expression and MMP-9 promoter activity (Fig 6), which was reversed by TG2 siRNA [159]. Furthermore, the authors suggest that TG2 diminishes c-jun – c-fos interactions and their recruitment to the AP-1 binding sites [159]. Although there are some weaknesses in the study, the data clearly demonstrates that TG2 suppresses MMP-9 message, protein and promoter activity.

In addition to direct regulation of transcriptional processes by nuclear TG2, cytosolic TG2 may also indirectly regulate transcriptional processes. One of the pathways known to be activated as such is NF-κB pathway. It is not totally clear how TG2 activates NF-κB and it is definitely possible that more than one mechanism exists [162]. The current understanding of NF-κB activation by TG2 relies on the latter's ability to polymerize inhibitory subunit alpha of NF-κB (I-κB), through its transamidating activity [163] (Fig 6). As a result of this polymerization, NF-κB dissociates from the inhibitor and translocates to the nucleus where it activates transcription [163]. The authors found that TG2 inhibition prevents NF-κB activation [163]. Other reports supporting this hypothesis have been published [84, 164, 165]. In addition to its role in I-κB polymerization, TG2 might regulate the NF-κB pathway through different mechanisms [156]. Very recent data from Mehta and his colleagues show that a transamidating inactive mutant of TG2 was as effective as wild type in activating NF-κB transcriptional activity (Kapil Mehta, personal communication). Overall, there is compelling evidence showing that TG2 activates NF-κB; however the involvement of transamidating activity of TG2 in the activation of NF-κB pathway is yet to be established.

Cytosolic TG2 also regulates the activity of another important transcription factor: cAMP-response element-binding protein (CREB). The mechanism by which TG2 activates CREB is not yet completely clear. A study from our group showed that TG2 regulates CREB activity by increasing intracellular cAMP levels [166] (Fig 6). This increase was suggested to be the direct result of the transamidase-dependent activation of adenylyl cyclase [166]. Another study demonstrated that TG2 interacts with protein phosphatase 2 (PP2Aα), a protein that dephosphorylates and inactivates CREB, and targets it for degradation [167]. Although the authors show that the transamidating activity is necessary for CREB activation, they do not show whether it is necessary for PP2Aα degradation, therefore, they fell short of showing a causal link [167]. A third study suggests a completely different mechanism. Kang *et. al*. [168] argues that TG2 activates CREB by activating Akt signaling through its adrenergic receptor responsive G-protein function. The fact that three different

groups reported TG2-dependent CREB activation in different models is a strong indication of the importance of TG2 in the regulation of CREB activity. However, the mechanism of this regulation remains to be established.

11. Conclusions

Research on TG2 started approximately 50 years ago when a new reaction in liver extracts was discovered and the enzyme responsible for this reaction was named “transglutaminase” [22]. TG2 was considered solely as a transamidating enzyme until 1987 when its GTP binding and hydrolyzing activity was defined [31]. Since then, several new enzymatic functions of TG2 were defined and we are still learning the extent of its multifunctionality. Although we do not know the most prominent role of TG2, it appears that its role on the cell surface is starting to emerge as one of the most important functions. Surprisingly, the extracellular role of TG2 is mostly not transamidase activity dependent. This is in line with our and several other group's observation that the most important function of TG2 in the cell is not its transamidase activity, but other activities.

One of the most curious questions in the TG2 field is that why TG2 knockout mice survive if this protein has so many important roles. The fact that TG2 knockout animals have no major pathologies makes it very difficult to establish the most prominent function of TG2. However, it is becoming evident that TG2 is a regulator, rather than a crucial player, in the processes it is involved. Metaphorically speaking, it is a fine tuning knob rather than an on/off switch. In this functional capacity, cells can cope with the absence of TG2 even though it is involved in many processes.

Of the many cellular processes in which TG2 is involved, determining cell death/survival processes is obviously an important one (Fig. 7). Depending on which function of TG2 is involved it can facilitate survival or death. In addition to different functions; localization and conformation state of the protein are also very important in determining the final outcome. In the past TG2 was considered to be a pro-cell death protein, primarily because in severely stressed models TG2 facilitated apoptosis [101, 169] and is upregulated in a variety of central nervous system pathologies such as chronic progressive neurodegenerative disorders [Huntington disease, Alzheimer disease, Parkinson disease and progressive supranuclear palsy] [109] or acute brain injury [170] and stroke models [171]. However, recent studies from our group [36, 114, 115] and others [49, 125, 165, 172] are causing a paradigm shift in the field. The new paradigm tends to depict TG2 more as a prosurvival protein. It is possible to speculate that the observed upregulation of TG2 in neurodegenerative conditions may be compensatory; i.e., an attempt by the cell to increase its chances of survival under stress. In addition to an upregulation of TG2, an increasing numbers of stressors are being reported to elevate the amount of TG2 in the nucleus [14, 71, 157]. We [114, 115] and others [84] have shown that hypoxic/ischemic stress very significantly increases nuclear TG2 amounts. It can also be speculated that accumulating TG2 in the nucleus is the cell's response to stress to cope with it. Given this, an important question to be addressed is how TG2 get into the nucleus. The mechanisms by which TG2 regulates transcriptional processes also remain to be elucidated. Overall all it is clear that TG2 plays a complex, yet important role in regulating cell death and cell survival decisions and though much is known much remains to be learned.

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Highlights

>Transglutaminase 2 (TG2) is a multifunctional protein

> TG2 undergoes extreme conformational changes and exhibits localization diversity

> A prominent function of TG2 is the regulation of transcription

> The role of TG2 in cell death processes is dependent on many variables

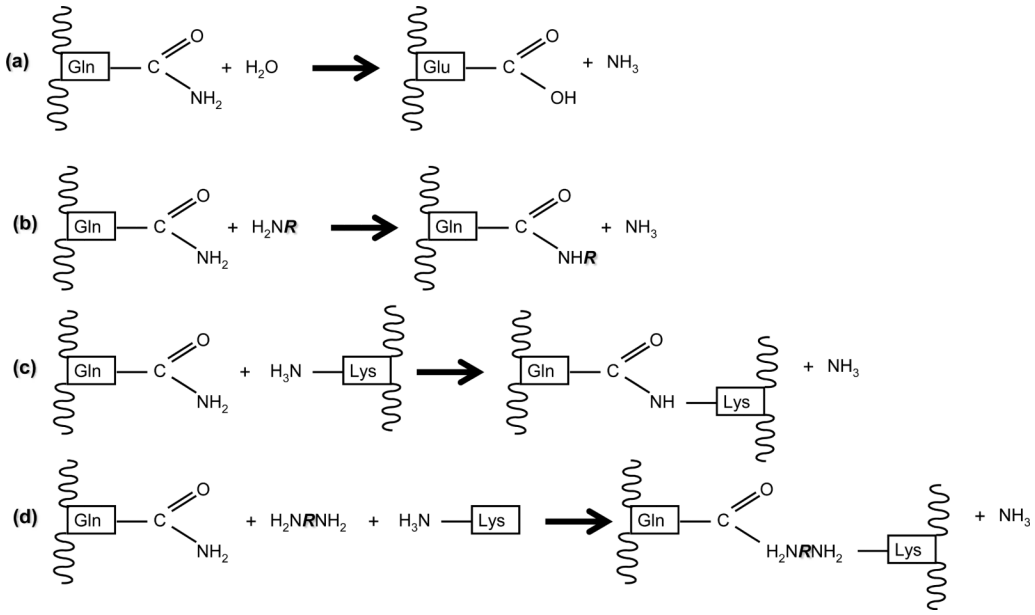

Figure 1. Reactions catalyzed by the transamidase activity of TG2

(a) TG2 can use a water molecule as an acyl-acceptor to deaminate a peptide-bound glutamine residue and convert it to a glutamate residue. (b) When a primary amine acts as acyl-acceptor, the glutamine residue is modified resulting in a posttranslational modification. (c) An isopeptide bond forms when the ε-group of a peptide bound lysine is the acyl-acceptor resulting crosslinking of two proteins. (d) The crosslink does not have to be an isopeptide bond. A primary amine can act as a crosslinker between two proteins.

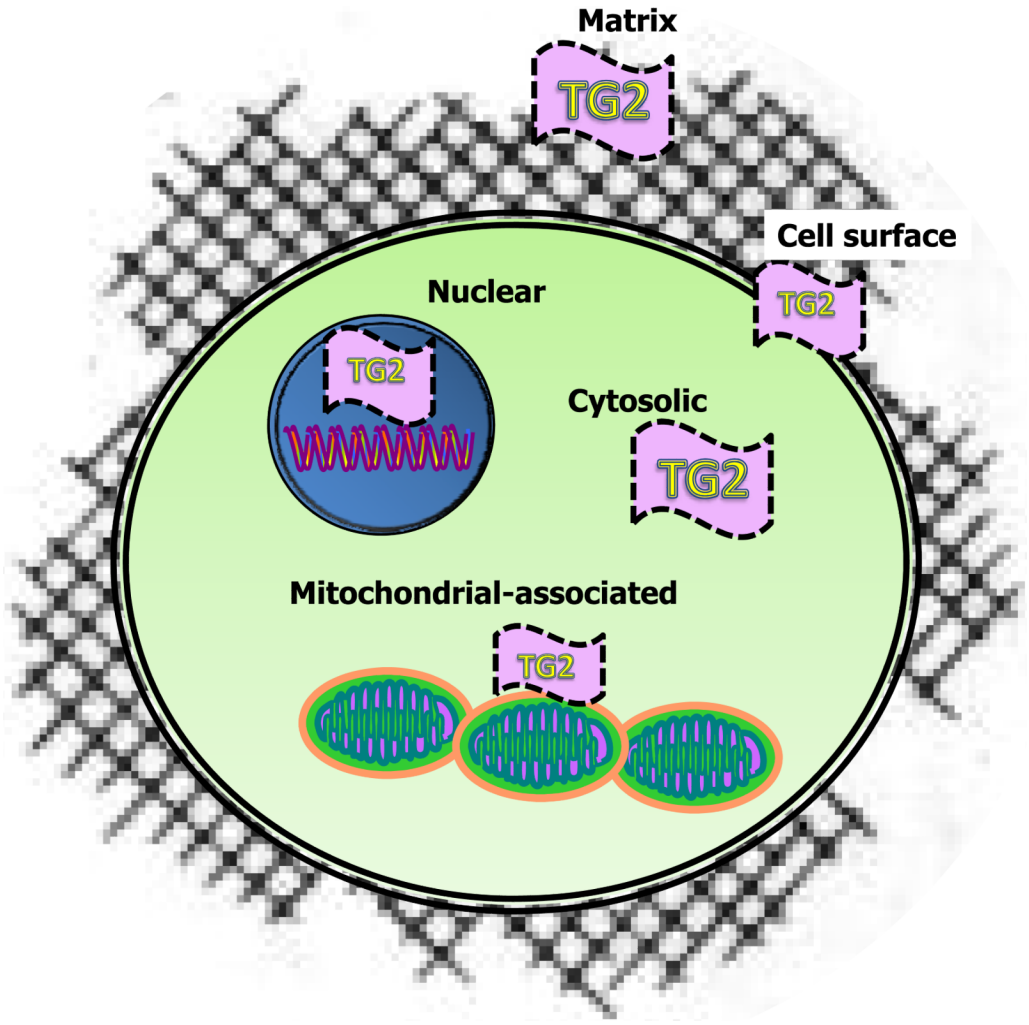

Figure 2. Localization of TG2 in the cell

TG2 has no known signal sequence, and is primarily localized in the cytosolic compartment. However, a small but significant portion of TG2 is localized in the nucleus, although the mechanism(s) by which it enters the nucleus has not yet been fully defined.. Although TG2 can modify mitochondrial enzymes, the evidence suggests that it is associated with mitochondria, but not found in the mitochondria. TG2 is also found outside of the cell, both associated with the plasma membrane and deposited into the matrix.

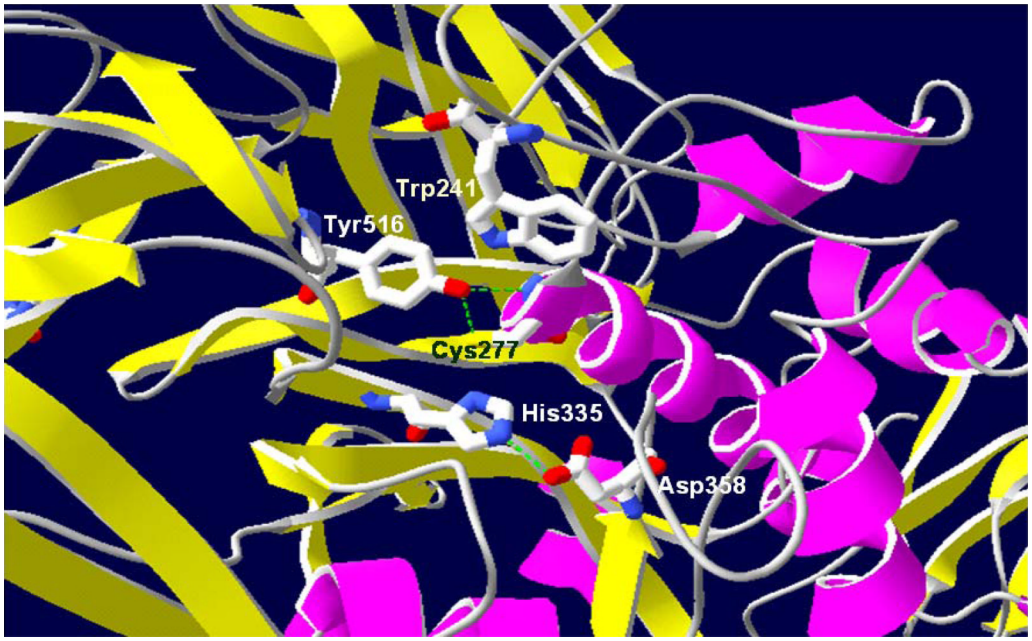

Figure 3. Transamidase active site of TG2

The catalytic site of transamidating activity is composed of the catalytic triad: Cys277, His335 and Asp358. A conserved tryptophan residue, Trp241 is also critical for the transamidating activity. A hydrogen bond forms between Cys277 and Tyr516 in the closed conformation of TG2, which is believed to further stabilize the closed conformation and keep the enzyme inactive.

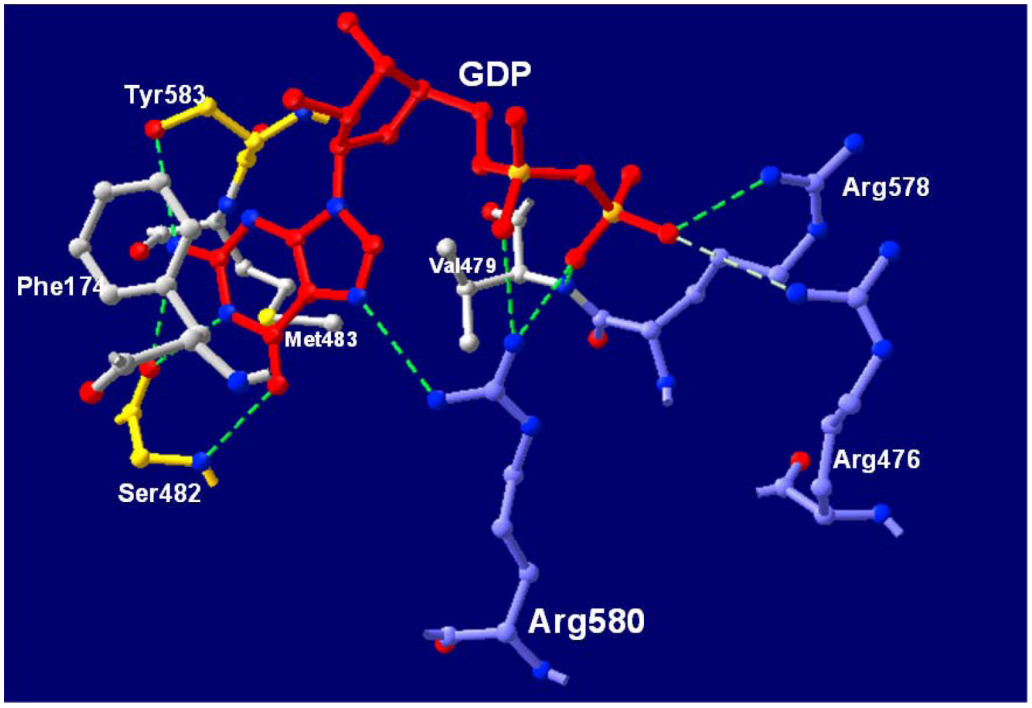

Figure 4. Guanine nucleotide binding pocket of TG2

The guanine nucleotide binding pocket is an example of a tertiary pocket, with contributing residues from the catalytic domain, β-barrel-1 and β-barrel-2 domains. Arg580 interacts with the guanine nucleotide at several points; loss of this residue significantly impairs GTP/GDP binding activity and disinhibits transamidase activity. Dashed green line depicts optimal H-bonding distance; dashed white line depicts suboptimal H-bonding distance.

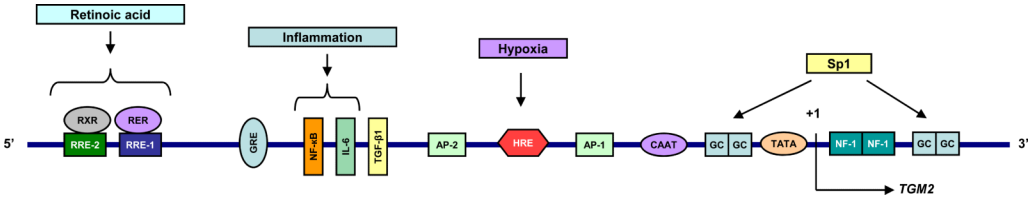

Figure 5. Regulatory elements of the human *TGM2* gene expression

The expression of TG2 is regulated by many factors. In this figure identified elements that regulate TG2 expression are shown. Retinoic acid response elements: RRE-1 (-1731), RRE-2 (-1720), glucocorticoid response element: GRE (-1399), nuclear factor κB response element: NF-κB (-1338), interleukin-6 response element:IL-6 (-1190), tumor growth factor-β1: TGF-β1 (-900), activator protein-2: AP-2 (-634), hypoxia response element: HRE (-367), activator protein-1: AP-1 (-183), CAAT box (-96), GC box: Sp1 binding motifs (-54, -43, +59, +65), TATA box (-29), nuclear factor-1: NF-1 (+4, +12).

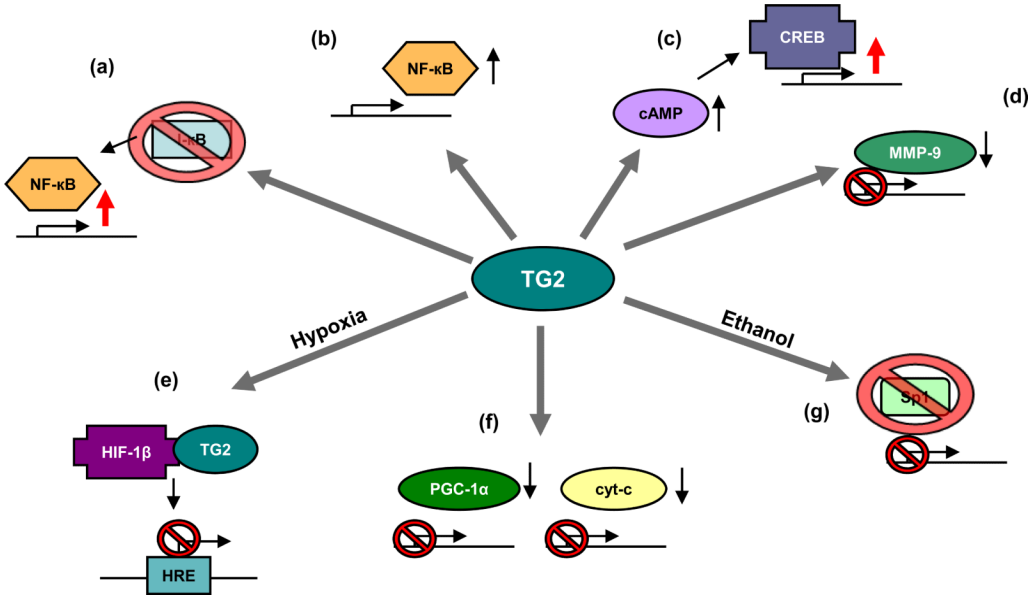

Figure 6. Transcriptional regulation by TG2

(a) TG2 transamidates I-κB, leading to NF-κB activation. (b) TG2 activates NF-κB transcriptional activity. (c) TG2 increases cAMP, leading to activation of CREB. (d) TG2 downregulates MMP-9 expression and promoter activity. (e) TG2 suppresses HRE activity in response to hypoxia. (f) TG2 suppresses PGC-1α and cyt-c expression. (g) TG2 crosslinks Sp1 in response to ethanol and downregulates Sp1 regulated gene transcription.

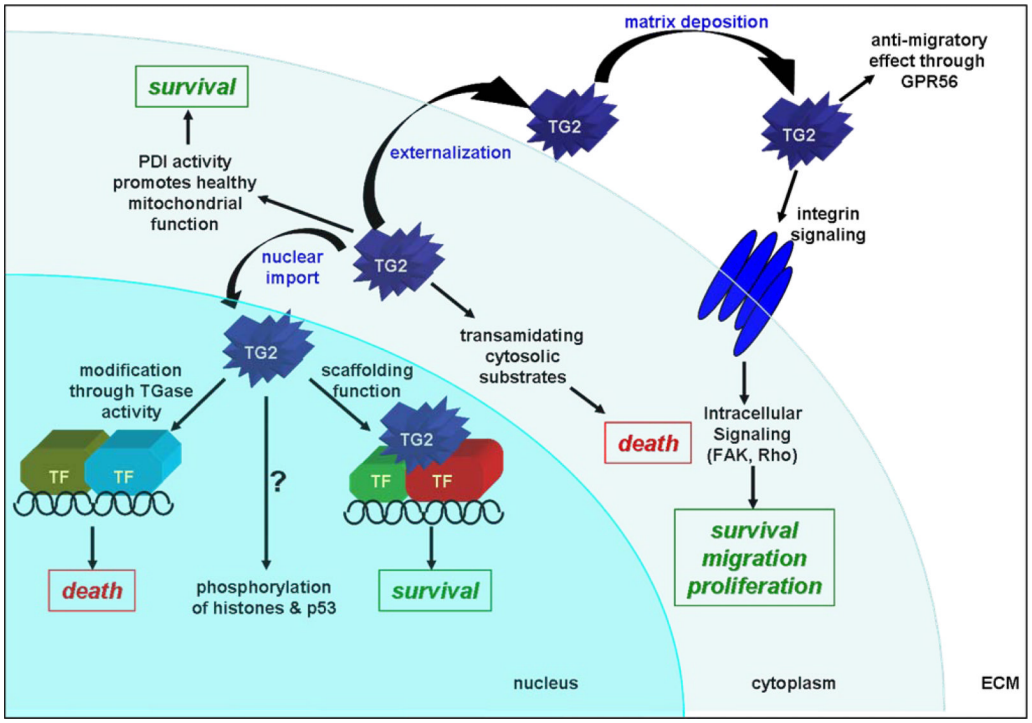

Figure 7. Simplified hypothetical model showing different roles of TG2 in death/survival paradigm

The complex circuitry of the cellular processes regulated by TG2. Depending on its localization, conformation and activity state, TG2 can differentially modulate cell death/survival processes. Relatively well described mechanisms that are widely exploited by TG2 to control cell death/survival processes are: *(i)* mediating cell to ECM contacts, thereby triggering cell survival signaling; *(ii)* controlling the activities of transcription factors directly or indirectly to promote survival or facilitate demise.