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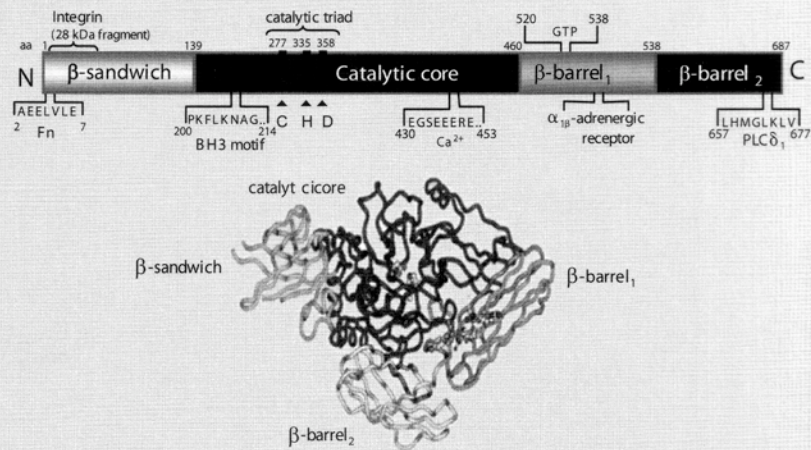
# Transglutaminases

## Family of Enzymes with Diverse Functions

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## **Type 2 Transglutaminase and Cell Death**

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Transglutaminases (TGases) are a large family of calcium-dependent transamidation acyltransferases, catalyzing post-translational modification of proteins. In invertebrates, only a single transglutaminase gene is known, whereas nine genes, evolutionary related and probably evolved by successive duplications have been identified in mammals [1]. In the present article, we will focus on the role of type 2 (TG2) or 'tissue' transglutaminase, first identified in 1953 as the liver enzyme incorporating amines into proteins [2]. In particular, we will focus on the potential role(s) played by the enzyme in cell death both under physiological and pathological conditions. TG2-mRNA expression and protein levels increase significantly in a dose- and time-dependent manner in response to cell death induction both in tissues and cell culture (fig. 1); TG2 expression can be used as a trace marker for detection and quantification of apoptosis in vitro and in vivo [2, 3]. The TG2-dependent cross-linking activity represents together with the extensive proteolysis (i.e. due to the activation of cellular cysteine proteases), the most important event leading to the modification of the proteome of a dying cell.

### **'Tissue' Transglutaminase or Type 2**

Tissue or type 2 transglutaminase (TG2) is the most ubiquitous isoform belonging to TGases family. TG2 is a versatile multifunctional protein

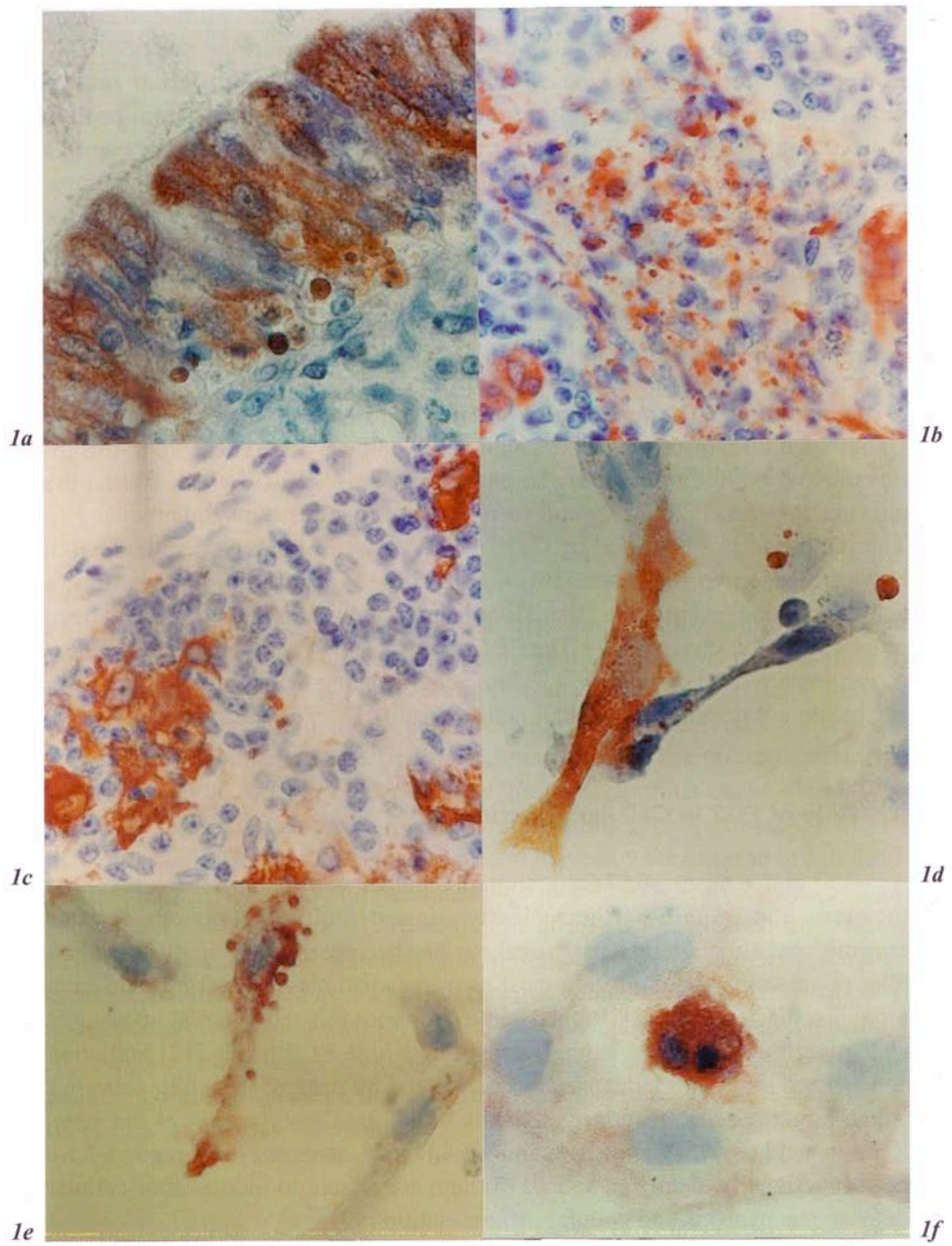
involved in a variety of biochemical functions at various cellular locations [2–4]. Depending upon the nature of the group entering in the reaction, TG2  $\text{Ca}^{2+}$ -dependent cross-linking activity is responsible for different related modifications such as incorporation of amine into proteins, protein-protein cross-linking as well as site-specific deamidation (if the entering group is a water molecule instead of an amine) [2]. Mainly under pathological settings, TG2 can also act extracellularly, it can be exposed on the external leaflet of the plasma membrane or released from cells, where it has been suggested to mediate the interaction between integrins and fibronectin with extracellular matrix (ECM) [5, 6].

In addition to the above mentioned activities, TG2 can act as G-protein binding and hydrolyzing GTP with an affinity and a catalytic rate similar to the  $\alpha$ -subunit of large heterotrimeric G proteins and small Ras-type G proteins [7]. Under such circumstances, TG2 couples  $\alpha_{1b}$  and  $\alpha_{1d}$  adrenoreceptors, thromboxane and oxytocin receptors to phospholipase C (PLC- $\delta_1$ ) [8]. When the enzyme is in a GTP/GDP-bound form, it cannot act as transglutaminase [9]. The inhibition is suspended by  $\text{Ca}^{2+}$  which plays a role as molecular switch between these two functions [10]. Finally, based on in vitro observations, a  $\text{Ca}^{2+}$ -independent protein disulfide isomerase (PDI) function has been proposed for this enzyme [11]. The PDI activity seems to rely on an independent active domain from that used for TGase activity, being the cysteine of this latter active site not responsible for PDI activity itself [11].

### **Role of TG2 in Cellular Functions**

The above described TG2 activities together with its various subcellular locations and protein partners clearly suggest multiple functions for this enzyme, and although, extensive analysis has been carried out in different cellular populations under physiological as well as pathological settings lacked to provide a unified view [2]. In fact, some cell types (e.g. endothelial, mesangial and smooth muscle cells) express constitutive high TG2 levels [12] and other cell types do not, and its expression is induced by distinct signaling pathways which target specific response elements in the regulatory region of the gene. Retinoic acid (RA), TGF- $\beta$ , IL-6 and NF- $\kappa$ B responsive sites and regions have been functionally identified and all of them are related to induction of cellular defense mechanisms and cellular differentiation [13].

Once expressed, the regulation of TG2 activities is mediated by multiple heterogeneous factors. As a G-coupled protein, the role of TG2 in transmitting signals from seven transmembrane-helix receptors to phospholipase C has been clearly described [9], being phospholipase C activated following TG2 binding of

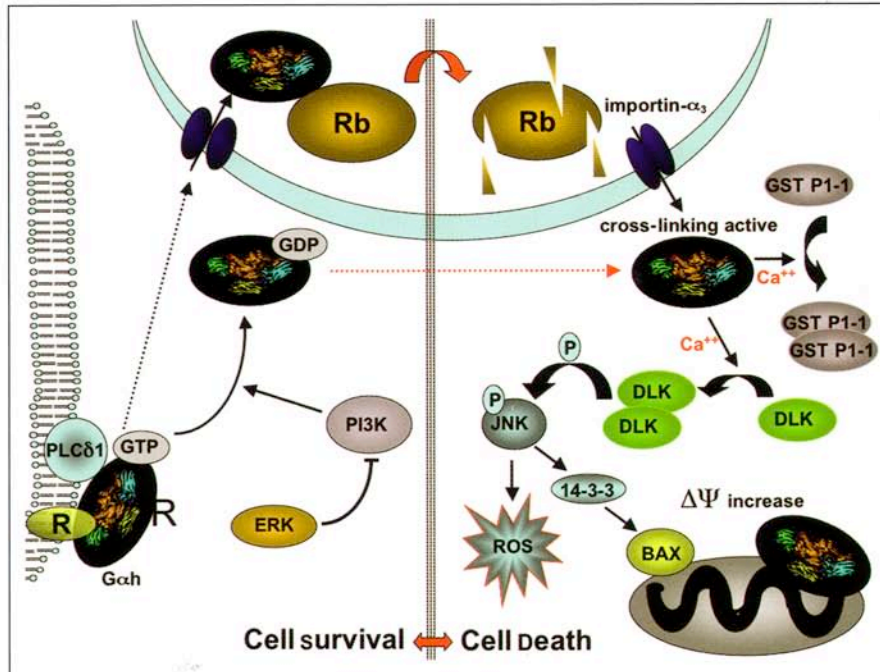


GTP (fig. 2). High  $\text{Ca}^{2+}$  levels can induce the release of GTP/GDP molecules, inhibiting signaling and promoting the transamidating activity [4, 9]. The  $\text{Ca}^{2+}$  requirement for such an activity might be reduced by the interaction of TG2 with specific molecules, such as sphingosylphosphocholine, [14, 15]. Nitric oxide can also strongly influence TGase activity, since up to 15 of the 18 cysteine residues in the protein can be nitrosylated and denitrosylated in a  $\text{Ca}^{2+}$ -dependent manner, inhibiting and activating the enzyme respectively [16]. Following activation by calcium, TG2 interacts and modifies major component of cytoskeleton [2–4]. After RA induction, the enzyme can modify RhoA, a member of Rho GTPases widely involved in cytoskeletal rearrangements [17, 18]. Such modification results in increased binding of RhoA to ROCK-2 protein kinase, autophosphorylation of ROCK-2 itself and consequent phosphorylation of vimentin, finally leading to the formation of stress fibers as well as increased cell adhesion [17]. Moreover, TG2 can interact with  $\beta$ -tubulin and with microtubule binding proteins, including tau, which can be eventually cross-linked by the enzyme [19, 20].

Cytoskeletal protein does not represent the only intracellular target for the transamidating TG2 enzymatic activity. The enzyme possesses nuclear localization sequences (NLS), TG2 can translocate, presumably with the help of importin-  $\alpha_3$ , into the nucleus (fig. 2) [21]. There, it can perform as a G-protein as well as transamidating enzyme, activated by nuclear  $\text{Ca}^{2+}$  signals to cross-link both histones and transcriptional factors [22, 23].

TG2 is selectively expressed in dying cells during programmed cell death *in vivo* (fig. 1 and 2). Its overexpression potentiates apoptosis and conversely, silencing through antisense technology or inhibition reduces the cell death onset [24]. The activation of TG2 transamidating activity in dying cells induces extensive polymerization of intracellular proteins, including actin, histones and retinoblastoma protein (Rb; 24–25) leading to the assembly of detergent insoluble structures [26]. These protein scaffolds stabilize the structure of the dying cell prior its clearance by phagocytosis [27]. TG catalyzed cross-linking limits,

**Fig. 1.** *In vivo* and *in vitro* immunohistochemical localization of TG2 expression in cells undergoing cell death. **a** Rat uterus 4 h postpartum; **b** Involuting mouse thymus 24 h after glucocorticoid injection; **c** HIV-infected human lymph nodes showing TG2-positive dying syncytia; **d** 48 h retinoic acid-treated human neuroblastoma cells; **e** Macrophage-derived HIV-infected human syncytia; **f** Higher magnification of retinoic acid-treated human neuroblastoma cells. Note the specific expression of TG2 (red staining) in cells showing the features of apoptotic cells (i.e. nuclear and cytoplasm condensation, chromatin margination and detachment from neighboring cells) as well as in dying syncytia. TG2 localization was detected both in 6  $\mu\text{m}$  of paraffin-embedded tissue sections (**a–c**) and in cell lines (**d, f**) using an affinity purified monospecific IgG raised in rabbits against human red blood cell soluble TG2.



**Fig. 2.** Schematic view of the prosurvival and proapoptotic TG2 functions. The cross-linking inactive TG2/G $\alpha$ h protein bound to various membrane receptors (R) can translocate into the nucleus where it protects Rb antiapoptotic transcriptional activity. The inhibitory effect of PI3K is inhibited by the growth factors-dependent continuous stimulation of the Ras-ERK pathway. As a consequence of various apoptotic stimuli the cross-linking active cytosolic TG2 post-translationally modifies DLK leading the activation of the JNK pathway that controls the translocation of BAX [74] on the mitochondria and consequently the activation of the intrinsic apoptotic pathway [74]. TG2 itself is also localized on mitochondria [28] and through the cross-linking of GST P1-1 [20] and of other mitochondrial substrates contributes by the alteration of the MMP and the formation of ROS to cell death. An apoptotic stimulus can lead to the detachment of Rb from TG2, its translocation to the cytosol where together with the other cytosolic protein, upon calcium activation, can act as proapoptotic enzyme.

the leakage of intracellular components, thus preventing the release of soluble, harmful and immunogenic cellular degradation products to avoid the occurrence of inflammatory and autoimmune reactions [27].

Finally, it has been recently reported that TG2 interacts with mitochondria; its localization on this organelle changes the intermembrane polarity status, shifting to a higher polarized one (fig. 2) [28]. Being mitochondria responsible for intracellular calcium buffering and being this ability related to mitochondrial

membrane potential, it has been hypothesized that activation of TG2 cross-linking activity might be the outcome of the described alterations [15, 28]. Overexpression of TG2 in neural cells determines the unbalance of the redox status of the cells leading to the accumulation of reactive oxygen species (ROS) associated to a large depletion of GSH [28]. In keeping with these findings, GST P1-1 acts as a very efficient acyl donor as well as acceptor TG2 substrate both in cells and in vitro (fig. 2) [20]. The TG2-dependent polymerization of GST P1-1 leads to its functional inactivation and is effectively inhibited by GSH [20]. Many genes involved in apoptosis encode proteins that may generate or respond to oxidative stress [29]. Massive GSH depletion characterizes the early phases of apoptosis and the fact that GST P1-1 might be functionally inactivated by TG2-catalyzed oligomerization indicates a potential proapoptotic role for TG2 in antagonizing the cytoprotective effect involving the elimination of ROS originating from oxidative metabolism [29, 30].

### **Effects of TG2 Deletion in Mice**

Although it is now clear that TG2 biochemistry has multiple and unique cellular features, knockout (KO) mice carrying the homozygous deletion of TG2 gene on a mixed background do not exhibit an embryonic lethal phenotype; they are viable and born with Mendelian frequency [31, 32]. Under normal physiological conditions TG2 KO animals do not show major anomalies [31–32]. Moreover, no obvious alterations have been observed in the onset of apoptosis, in the ECM structure or in the heart function, in which TG2 G-protein activity is thought to be important [31, 32]. However, when cell death is induced in vivo in KO mice, the clearance of apoptotic cells by phagocytosis is defective in the thymus and in the liver and inflammatory as well as autoimmune reactions develop in the animals [33]. The most obvious explanation for the lack of lethal phenotypes is that other transglutaminases may exert a compensatory effect in mammalian tissues. However, such compensation is necessarily partial, since there are no evidences showing that the other mammalian transglutaminases can bind GTP acts as PDI or, with the exception of FXIIIa, be exposed out of the cell surface. Thus, it is reasonable to suppose that, at least under certain pathological or stressful conditions, evidences for organ alterations could be gained from more careful studies [34]. In fact, consistent with the described extra- and intracellular functions of TG2, decreased adherence of primary fibroblasts and impaired wound healing, related to altered cytoskeletal dynamics in fibroblasts, have been observed in TG2 KO mice [32]. Finally, it has been shown that TG2 deficient mice have impaired insulin secretion with consequent glucose intolerance and hyperglycemia, a phenotype

strongly resembling a subtype of diabetes named MODY (for maturity-onset diabetes of the young) [35].

### **Is TG2 a Proapoptotic or Prosurvival Protein?**

In the last 15 years more than 300 papers have clearly shown that TG2 acts as a proapoptotic enzyme. In keeping with this notion, TG2 KO mice show defects in the clearance and stability of apoptotic cells [33]. However, recently antiapoptotic activities for the enzyme have also been reported [36–38]. TG2 is mostly cytosolic, however the enzyme protein is also localized in the nucleus and associated with the plasma membrane (fig. 2) [38]. It has been proposed that both the intracellular localization and the transamidating activity of TG2 are important in modulating its effects on apoptosis [38]. In fact, cells transiently transfected with wild-type TG2 or mutants (which lack transamidating activity) targeted to different intracellular compartments confirmed the proapoptotic nature of cytosolic TG2 [38]. By contrast, the nuclear localization of cross-linking-inactive TG2 reduced apoptosis [38]. Membrane-targeted TG2 had neither pro- nor antiapoptotic functions, thus indicating that intracellular localization is essential in determining the effect of TG2 on cell death. Although, the early induction of TG2 in dying cells in tissue suggests regulatory function for the enzyme, the molecular mechanisms of its action are only recently being partially unveiled (fig. 2).

DAP-like kinase (DLK) is a nuclear serine/threonine-specific kinase, which has been implicated in apoptosis [39]. This protein belongs to a subgroup of serine/threonine protein kinases, referred to as the mixed-lineage kinases, that act as key regulators of the stress-activated c-Jun N-terminal kinase (JNK) mitogen-activated protein kinase signaling pathway [40]. Induction of apoptosis by DLK requires its relocation to the cytoplasm, in particular its association with the actin cytoskeleton which is achieved through interaction with proapoptotic protein Par-4 [41]. DLK undergoes TG2-dependent oligomerization in cells undergoing apoptosis upon exposure to calphostin C (fig. 2) [39]. Overexpression of DLK in neural cells and in sympathetic neurons induces apoptosis through the mitochondrial-dependent death pathway; conversely, overexpression of a dominant-negative form of DLK in these cells prevents apoptosis, thus indicating that DLK is involved in the control of cell death [42]. Similarly with the suggested TG2 dual function, also nuclear DLK does not induce apoptosis and rather it has been implicated in transcription [43]. Interestingly, the TG2-dependent DLK oligomerization occurs early in the apoptotic response and significantly enhances the kinase activity of DLK and consequently its ability to activate the JNK pathway (fig. 2) [39]. Moreover,



functional studies demonstrate that TG2-mediated oligomerization of wild-type DLK sensitizes cells to calphostin C-induced apoptosis, while cross-linking of a kinase-inactive variant of DLK does not [39]. These findings suggest that TG2 proapoptotic activity is at least partially mediated by the oligomerization and activation of the proapoptotic kinase DLK that in turn will activate the proapoptotic c-Jun N-terminal kinase pathway (fig. 2).

As far as the antiapoptotic mechanisms of TG2 are concerned only recently some clues about the possible mechanisms at the basis of this prosurvival function have been published [36–38]. We first demonstrated that TG2 post-translationally modified the Rb, an important suppressor of apoptosis [22]. Recently, it has been suggested that the TG2 interaction with Rb increases significantly concomitant with an attenuation of apoptosis (fig. 2) [38]. Thus implying that TG2 might protect cells against apoptosis in response to stimuli that translocate the enzyme into the nucleus and do not result in increased transamidating activity [38]. The TG2/Rb interaction is emerging as an important aspect of the prosurvival effects of TG2. In fact, while in cells undergoing apoptosis Rb is degraded by caspases, this degradation is blocked when cells are pretreated with RA, an important transcriptional inducer of TG2 (fig. 2) [37]. Experiments performed with Rb(–/–) fibroblasts demonstrate that Rb is required for TG2 to exhibit antiapoptotic activity in response to RA treatment [37]. Thus suggesting that the ability of TG2 to modify Rb represents a key step for TG2 to provide protection against apoptotic insults and to ensure that cells remain viable. However, it is still controversial whether the transamidating activity of nuclear TG2 is required to bind and protect Rb from the degradation occurring during apoptosis [37, 38].

It is well known that RA consistently induces TG2 expression and activation [44]. It has been shown that RA-dependent TG2 expression protects NIH3T3 cells from apoptosis [45]. Interestingly, in this cell line RA stimulation activates ERK and phosphoinositide 3-kinase (PI3K); however, only PI3K activation is necessary for RA-induced TG2 expression (fig. 2). The exposure of cells expressing TG2 to the PI3K inhibitor reduces the ability of the enzyme to bind GTP, suggesting that PI3K might regulate the GTP binding activity of TG2 consequently, blocking its transamidating activity (fig. 2) [45]. Moreover, cell viability assays showed that incubation of RA-treated cells with LY294002 together with the TG2 inhibitor monodansylcadaverine convert RA from a differentiation factor to an apoptotic stimulus [45]. These findings suggest that PI3K activity is required for the RA-stimulated expression and GTP-binding activity of TG2, thereby linking the upregulation of TG2 with a well-established cell survival factor (fig. 2). In keeping with this notion, TG2 expression and its GTP-binding activity is very high in a number of transformed cell lines indicate that constitutively active TG2 may be a characteristic of certain cancer cells [46]. These findings suggest that

TG2 may also act as a survival factor and its expression/activation requires the PI3K, but is antagonized by the Ras-ERK pathway. Interestingly, the switch between the prosurvival TG2 GTPase activity and the cell death transamidating one can be achieved through the Ras-ERK pathway [46].

Future studies should address as to how the different catalytic activities of the enzyme are involved in cell survival or as to whether this antiapoptotic function is simply achieved by protein-protein interactions and by which partners.

### **Is TG2 Involved in Autophagy?**

Autophagy is a degradative mechanism mainly involved in the recycling and turnover of cytoplasmic constituents from eukaryotic cells [47]. Recently, genetic screenings have considerably increased our knowledge about the molecular mechanisms of autophagy identifying number of genes involved in fundamental steps of the autophagic pathway [48]. In yeast, autophagy is mainly involved in adaptation to starvation, but in multicellular organisms this route is emerging as a multifunctional pathway involved in a variety of additional processes such as autophagic cell death (ACD), removal of damaged organelles and development of different tissue-specific functions [49]. In addition, autophagy is involved in a growing number of pathological conditions, including cancer, myopathies and neurodegenerative disorders [47]. The existence of a mitochondrial-specific autophagic programme has been proposed [50], this is suggested by the observation that a Bcl-2 antisense oligonucleotide can trigger mitochondrial membrane permeabilization (MMP) and ACD [50]. In several paradigms of ACD induction, the so-called death-associated protein (DAP) kinase has been shown to be required [51]. Overexpression of constitutively active DAP-kinase is sufficient to trigger ACD accompanied by MMP [50, 51], and Bcl-2 can prevent DAP-kinase-induced cell death, presumably through its capacity to interfere with MMP [50, 51]. These findings further indicate the existence of a crosstalk between autophagic and apoptotic cell death; recent findings indicate that multiple genes involved in apoptosis are also acting during ACD [52], supporting the notion that these two processes can utilize common pathways or pathway components. As previously reported, TG2 regulates the functions of DLK that is one of the member of the DAP-kinase family (fig. 2) [47]. Based on these findings and considering its pathogenic role in neurodegenerative diseases (see below) it is possible to hypothesize that, under some circumstances, TG2 could be one of the molecule participating in the physiological switch between ACD and apoptosis [53]. It is also relevant to mention that the persistence of the death stimulus and the impairment of the mitochondrial function in the presence of an apoptosome block can lead to death by an alternative pathway inducing

autophagy [50]. In keeping with these notions, TG2 overexpression in various cell lines leads to their priming for cell death switching from a caspase-dependent into a caspase-independent type of death [20, 28], thus further suggesting its potential pro-ACD function. In fact, autophagic activity remained elevated in neurons treated with pan-caspase inhibitor, which inhibited morphological apoptosis but neither inhibit cytochrome C release nor prevent cell death [54]. Future studies should define as to whether TG2 is indeed involved in this cell death switch under physiological and pathological settings.

## **TG2 and Cell Death in Pathological Settings**

### *Neurodegenerative Diseases*

As an active component of the cell death machinery, TG2 is involved in the pathogenesis of several diseases, the enzyme being activated in various disorders [2, 27, 33–35, 55]. TG2 cross-linking activity has been proposed to participate to protein aggregates formation occurring in the major neurodegenerative diseases, including Alzheimer, Parkinson and Huntington's Disease (HD) [56, 57]. In particular, HD is a progressive autosomal-dominant neurodegenerative disorder (incidence 1 in 10,000 individuals), resulting from expanded polyglutamine tracts (beyond 35–40 residues) in the coding region of huntingtin (htt) [58]. The disease is associated with the formation of misfolded ubiquitinated protein aggregates, (neuronal intranuclear inclusions; NII), containing all or part of the mutant htt. These protein inclusions have predominantly been detected in the nucleus, but may also be observed in the cytoplasm of striatal and cortical neurons [58]. Several reports suggest that in vitro aggregation might occur both through polar zippers formation and/or protein cross-linking catalyzed by TG2 [56, 59]. On the other hand studies on cellular models showed that TG2 does not interact with mutant htt in vitro [60]. In order to elucidate the role of TG2 in HD, we produced a new animal model, by crossing a TG2 KO strain [31] with the R6/1 strain, transgenic for exon 1 of htt carrying 116 (CAG) repeats, which develops a HD resembling phenotype [61]. The transgene expression induces a marked increase in the  $\epsilon(\gamma\text{-glutamyl})\text{lysine}$  bond levels, as compared to controls, thus indicating that the polyglutamine tracts are good TG2 substrates in vivo [62]. In keeping with these findings, the depletion of TG2 in HD transgenic mice results in a drastic reduction in  $\epsilon(\gamma\text{-glutamyl})\text{lysine}$  bonds [62]. The drastic decrease (10-fold) of the isodipeptide levels, observed in the TG2-null mouse brain, represents the first in vivo evidence of the central role, among the various members of the transglutaminases family, played by TG2 in mouse brain [62]. Interestingly, ablation of TG2 did not produce a significant reduction in NII number, thus suggesting that TG2 cross-linking of the mutated ntt is not directly involved in NII assembly

in vivo. By contrast, a significant increase in the NII/nuclei ratio in R6/1, TG2<sup>+/+</sup> versus R6/1, TG2<sup>-/-</sup> brains was observed, suggesting that TG2 gene deletion causes impairment in some protective mechanism preventing the assembly of these nuclear protein complexes [62].

As previously mentioned, TG2 gene induction characterizes cells undergoing programmed cell death in either physiological or experimental settings (fig. 2). In previous studies, other groups [63] and our own [53] described that cell death in the cortex and striatum of HD-transgenic mice is characterized by condensed neurons not displaying the classical apoptotic features, rather these dying cells have been shown to undergo ACD. Interestingly, a dramatic reduction (60–70%) in the number of these dying cells in the neocortex and striatum of HD transgenic/TG2 KO brains, as compared to HD transgenic animals, was observed [62]. It is important to note that the TG2 gene deletion ameliorates the neurodegenerative process observed in HD mice both in the symptoms and survival of these animals with a significant increase of their life span [62]. These evidences indicate that TG2 is the most relevant protein cross-linking enzyme among the various members of the TG family expressed in mouse brains and that TG2 plays an important role in the cell death process characterizing HD pathogenesis.

### **Liver Pathologies**

TG2 has been shown to have an important role in the maintenance of tissue integrity following cell stress or injury [2, 64]. Its role in tissue repair, as a response to loss in tissue homeostasis following trauma, is well documented [64]. The participation of the enzyme has been proposed in various degenerative diseases leading to severe tissue damage characterized by cell death and accumulation of insoluble protein aggregates both at intracellular and extracellular level [64, 65]. These diseases include, between others, various types of liver damages. A liver pathology characterized by accumulation of intracellular aggregate is the alcoholic hepatitis. The characteristic of this disease is the formation of Mallory Bodies that are cytoplasmic deposits of cytokeratin proteins, and TG2 has been shown to participate in their cross-linking [66].

In hepatic diseases characterized by fibrotic tissue formation, TG2 has been shown to participate in ECM cross-linking. In acute hepatic failure, such as in Budd-Chiari syndrome and in chronic liver pathology (viral hepatitis especially HBV and HCV), an abnormal accumulation of TG2 has been described on hepatocytes localized on the areas adjacent to scar and/or fibrotic tissue [34, 55, 67, 68].

At present, the liver fibrogenesis is referred to a dynamic process, strictly related to the extent and duration of parenchymal injury [69]. TG2 has been

suggested to play a role in the ECM organization either in normal or in pathological conditions [55]. A variety of ECM components, such as collagens, fibronectin, fibrinogen, laminin, nidogen and transforming growth factor- $\alpha_1$  (TGF- $\alpha_1$ ) act as TG2 substrates [5, 68, 70–72]. On the other hand, several inflammatory mediators, including TNF- $\alpha$ , TGF- $\beta$ , IL-1 $\beta$ , IL-6, involved in the fibrotic process and in apoptosis induction, are known to regulate TG2 expression in various biological settings [64].

The TG2 enzymatic reaction contributes to these pathologies by increasing the resistance of the deposited ECM to breakdown. Its ablation, in a mouse model, leads to an impaired liver regeneration after injury, associated with an increased inflammatory response, an abnormal tissue architecture and a reduced survival [34]. This evidence supports the hypothesis that the increased TG2 levels detected in early stages of HCV-induced liver damage plays a protective role, trying to maintain liver architecture [34]. The enzyme, by cross-linking extracellular proteins and/or by increasing cell-cell and cell-ECM adhesion, might also counteract the infiltration of inflammatory cells in the liver parenchyma. According with this view, TG2 induction must be considered as part of stress-induced damage and an important member of the hepatic tissue reaction to the progression of liver pathogenesis [34].

Another important function of TG2 during liver pathogenesis is its participation to apoptotic cell death. The abnormal accumulation of TG2 on hepatocytes in Budd-Chiari syndrome depends on the rapid induction of apoptosis that takes place in this disease [67].

The apoptotic cell death has been described in HCV-infected liver, but it is not a prominent phenomenon during this type of liver pathogenesis; in fact, when the liver activity increases, the HCV appears to have the ability to inhibit apoptosis advantaging its replication [73].

In general the enzyme has a protective and stabilizing role in hepatic damage, by eliminating the infected/altered cells and by contributing to tissue repair. However, under pathological condition, the uncontrolled activation of TG2 can turn its protective function to a pathological one. In some cases the stress response might be potentiated leading to pathological effects, in fact, excessive TG2 protein cross-linking can increase the resistance of ECM to breakdown and generate hepatic cirrhosis.

## Conclusions

In conclusion, despite extensive investigations, the question of the physiological role played by TG2 and the relevance of the post-translational modifications of its substrates remained largely unanswered so far. As discussed in

this review, compelling evidence demonstrated that the enzyme is involved in the regulation of cell death under physio-pathological settings [2]. However, several other questions remain to be addressed: is indeed the enzyme acting as PDI *in vivo*? Is this the physiological TG2 activity? Is this activity involved in the regulation of cell death? It will also be important to investigate as to how this potential enzyme activity complements the transamidating and G-protein TG2 activities. In fact, under stressful circumstances leading to increased free calcium concentration, such as those induced by many cell death stimuli, TG2 appears to switch its activity from the G-protein to cross-linking enzyme actively participating in apoptosis/autophagy (fig. 2). Does the PDI act on the same substrates modified by the TG2 during cell death?

TG2 represents a striking example of a cell death-associated protein playing different functions according to the cellular context and needs.

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