International Journal of Biochemistry Research & Review
4(1): 43-65, 2014

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History and Biology of Transglutaminase 2: A Synopsis

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Authors' contributions

Author OBO designed the study and wrote the first draft. Author PC did the literature searches. Both authors read and approved the final manuscript.

Review Article

Received 11th August 2013
Accepted 4th October 2013
Published 13th November 2013

ABSTRACT

Transglutaminase 2 (TG2) belongs to the family of transglutaminase, a large group of intracellular and extracellular enzymes that primarily catalyze the Ca^{2+}-dependent post-translational modification of proteins. Discovery of the first transglutaminase in the early 1920s, has subsequently lead to the identification of nine members of the enzyme family including TG2; the most abundant, most popular and most studied member of the transglutaminase enzyme family. The popularity of TG2 is due to its uniqueness amongst other members of the Transglutaminase (Tgase) family. Its difference from other Tgase family members is due to its specialized structural and biochemical activities; abundant tissue distribution and sub-cellular localization; and multi-functionality and physiology. The growing interests in TG2 and related research has resulted to an attendant mega-research output; and the need to produce a well-structured compilation of data on this popular enzyme has arisen. It is against this background, that we have compiled herein, a synopsis of available literature on TG2 history, structural and biochemical activities, tissue distribution, and physiology. This was done with the view to providing a compendium of background information that could be handy to researchers and new interest in the field of TG2 research.

Keywords: Transglutaminase 2; history; biology; structure; biochemistry; tissue distribution.

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1. INTRODUCTION

The human TGM2 gene localizes to chromosome 20q11-12 and its exons span approximately 37 kb [1]. The protein, transglutaminase 2 (TG2) is made up of 686 amino acids and has a calculated molecular mass of 76 kDa [2]; together with the first methionine, the molecular mass becomes 77.3 kDa and the amino acids 687 [1, 3]. However, following the transcription of TG2 in the free cytoplasmic space, it is N-terminally modified by the removal of the first methionine residue and the N-acetylation of the next to the last alanine residue [4]. Transglutaminase 2 (TG2) is also known as tissue transglutaminase (tTG), cytosolic, type II, or liver transglutaminase. It is a unique member of the transglutaminase (TGase) family of enzymes because in addition to its primary enzymatic activity of catalyzing the calcium-dependent posttranslational modification of proteins, it can also bind and hydrolyze GTP and may act as a G protein [5]. It also has a protein disulfide isomerase activity and may function as a protein kinase [6,7]. Besides acting intra-cellularly, TG2 can play some extracellular roles by taking part in cell adhesion processes and stabilization of the extracellular matrix [8]. It is the most abundant and most studied of the nine members of the transglutaminase enzyme family, including transglutaminase 1 (TG1), transglutaminase 3 (TG3) and transglutaminase 5 (TG5) isoforms, which are predominantly expressed in the epithelial tissue; TG4, which is expressed in the prostate gland; factor XIII (FXIII), which is expressed in the blood; transglutaminase 6 (TG6) and transglutaminase 7 (TG7), whose tissue distribution is unknown; and band 4.2, which is an enzymatically inactive component protein of the erythrocyte membrane that serves to maintain erythrocyte integrity [5]. After more than five decades since the discovery of the Transglutaminase family of enzymes, many reports have shown that the enzymes are ubiquitously present in microorganisms, plants, invertebrates and vertebrates (reviewed in Cai et al. [9]. Thus, the importance of the transglutaminase enzymes could be underscored by their wide distribution of the in all living organisms. In this article, we have presented a compendium of literature on the history and biology of TG2, with emphases on its uniqueness as evidenced by the structural and functional elements; biochemical multi-functionality, ubiquitous tissue distribution and sub-cellular localization, and substrate specificity, as well as involvement in many cellular physiologies.

2. THE TRANSGLUTAMINASE FAMILY AND THE ADVENT OF TG2

In the early 1920s, the reason behind the variation of fibrin solubility in urea was a matter of serious puzzle. In 1923, Barkan and Gaspar reported the cross-linking of fibrin polymers for the first time [10]; then, in 1948, Laki and Lóránd attributed the cross-linking to a Ca^{2+} - dependent protein called 'fibrin-stabilizing serum factor' or 'Laki-Lóránd factor' [11,12 and 13]. Subsequently, the 'serum factor' was purified by Loewy and colleagues [14]; and upon demonstration that hemophilia occurs as a result of its deficiency in the blood of hemophiliac patients, the enzyme was termed 'blood coagulation factor XIII [15]. In 1966, Lóránd*et al.* [16] observed that the 'blood coagulation factor XIII' was an isoenzyme belonging to the transglutaminase family. However, the term transglutaminase was first used by Waelsch and colleagues, while reporting the ability of a soluble liver protein fraction (containing TG2) to incorporate labeled amines into proteins in the presence of Ca^{2+} [17]. The designation, transglutaminase was later amended by the Enzyme Commission (EC 2.3.2.13, transglutaminase = R-glutamyl-peptide, amine-γ-glutamyltransferase). In 1987, Achyuthan and Greenberg [18] demonstrated the ability of transglutaminase 2 (TG2) to bind GTP with the resultant inhibition of its activity; justifying the reason why TG2 was named a G protein with a role in signal transduction [19].

In 1971, Harding and Rogers [20] identified gamma-glutamyl-epsilon-lysine cross-links in hair protein extracts; upon demonstration that this cross-linking enzyme was neither identical to factor XIII nor transglutaminase 2 [21], the enzyme was labeled an 'epidermal' or 'hair follicle' transglutaminase (TGe). Subsequently, it was observed that both membrane-bound and soluble fractions of the hair protein extract showed TG activity [22], hence, suggesting the presence of further epidermal transglutaminase. The insoluble, 'keratinocyte-specific' (corresponding to TGk) transglutaminase was detectable in cultured keratinocytes unlike the soluble 'epidermal' TGe [23]. Furthermore, the demonstration of the expression of TGk, TGc, and TGe in both hair follicle and epidermal keratinocytes [24], generated confusion and led to the numbering of transglutaminase isoenzymes and their corresponding genes [25, 26]; where the gene is designated with 'TGM' and the gene product is denoted by 'TG', both followed by an Arabic number. Thenceforth, TGM1/TG1, TGM2/TG2, and TGM3/TG3 were respectively assigned to TGk, TGc, and TGe; with their corresponding gene products. This system of naming did not alter the nomenclatures of factor XIIIa (FXIIIa) and band 4.2, and allowed for the classification of new TG family members that were subsequently discovered.

In subsequence, other transglutaminase have been discovered either by protein isolation or through sequence homology; hence, the isolation of TGp (TG4) from prostate adenocarcinoma cells by [27]; and more recently, Aeschlimann and colleagues have identified three new family members of TG: TGx (TG5), TGy (TG6), and TGz (TG7) [28, 29]. However, a catalytically inactive erythrocyte membrane protein band 4.2 was also discovered to belong to the TG family. Though, it has over 30% similarity with certain TG isoenzymes, a cysteine to alanine substitution within its active site made it catalytically inactive [30]. Today, a total of nine different transglutaminase isoenzymes have been identified in man.

3. STRUCTURAL AND FUNCTIONAL ELEMENTS OF TRANSGLUTAMINASE 2

The uniqueness of TG2 and the reasons for its multi-functionality can be found in its structure. Its basic structure is similar to those of other transglutaminase, but additionally, it bears some specific features which are not characteristic of other type of transglutaminase. Essentially, TG2 is structurally composed of four distinct globular domains (Fig. 1a): an NH2-terminal β-sandwich which contains fibronectin and integrin binding sites, a catalytic core which contains the catalytic triads (Cys277, His335 and Asp358) for acyl-transfer reaction and a conserved Tryptophan essential for this catalytic reaction [31], and two COOH-terminal β-barrel domain with the second barrel domain containing a phospholipase C binding sequence [32,33].

Unlike other transglutaminase, TG2 possesses a unique guanidine nucleotide-binding site, located in the cleft between the catalytic core and the first β-barrel (Fig. 1b.) [33]; this sequence is coded by exon 10 of the TG2 gene, which is characterized by very poor sequence homology with the same exons in other transglutaminase. Some GDP/GTP-interacting residues and those necessary for GTP hydrolysis are situated in other domains [34]. In the GDP-bound form of TG2, access to the transamidation active site is blocked by two loops, and the active site cysteine is attached to a tyrosine residue by hydrogen bonding. In the latent conformation of TG2, there is a significant inter-domain interaction between the catalytic domain 2 and domains 3 and 4, which reduces the accessibility of the active centre [33].

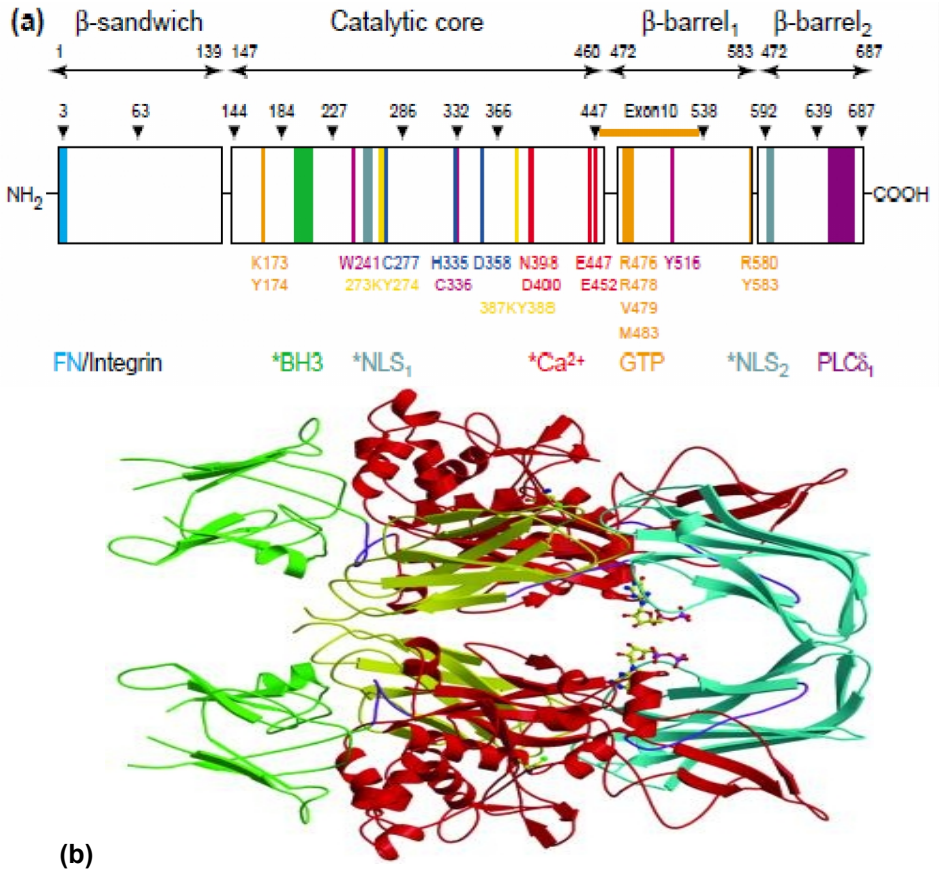

Fig. 1. Functional elements of transglutaminase 2 (TG2) and the overall structure of TG2 dimer with bound GDP [2,33]. (a) (a) The functional elements of TG2 indicating the four structural domains (arrows) and amino acid positions (top), with the different functional regions indicated as discussed above as reviewed by Fesus and Piacentini [2] (b) Overall structure of a transglutaminase 2 (TG2) dimer bound with GDP. TG2 is indicated as ribbon drawing with the β-sandwich domain, the catalytic core domain, and the first and second β-barrel domain shown in green, red, cyan, and yellow, respectively. The loops connecting the first β-barrel domain to the catalytic core and the second β-barrel are shown in purple. GDP is shown as a ball-and-stick model between the catalytic core and the first β-barrel as reviewed by Lui et al. [33].

The structural conformation of TG2 in its Ca^{2+}-bound form is yet to be resolved. A putative Ca^{2+}-binding site, homologous to the one demonstrated in FXIIIA by [35], is distorted in the TG2 structure by the bound nucleotide [33]. The binding of Ca^{2+} to the catalytic domain of TG2 alters the conformation of proteins through the removal of domains 3 and 4 further apart from the catalytic domain, thus making the active site of TG2 accessible [33, 36]; the hydrogen-bonded tyrosine is also displaced in the process [37]. The inhibitory implication of GTP on the transamidation activity of TG2 is mediated by GTP binding and subsequent hydrolysis involving Ser171 and Lys173 residues of the second domain [34].

4. TISSUE DISTRIBUTION AND SUB-CELLULAR LOCALIZATION OF TG2

The second reason for the uniqueness of TG2 is its involvement in diverse range of biological processes as a result of its wide tissue distribution and sub-cellular localization. Unlike other transglutaminase, the expression of TG2 is not restricted to only few tissues or certain cell types neither is it confined to a particular location in a cell [38]. Essentially, the cellular distribution of TG2 is ubiquitous, with its expression levels highest in endothelia cells and monocyte-derived macrophages; although, it is significantly expressed in vascular smooth muscle cells, connective tissue fibroblasts, osteoblasts, neurons , hepatocytes, astrocytes, and epidermal keratinocytes [2,5]. Transglutaminase 2 is constitutively expressed in different types of cells, while in some other cells its expression is induced by external stimuli or as part of their differentiation/maturation [39]. At cellular level, TG2 is localized both inside the cell and on the cell surface as shown by the schematic representation below:

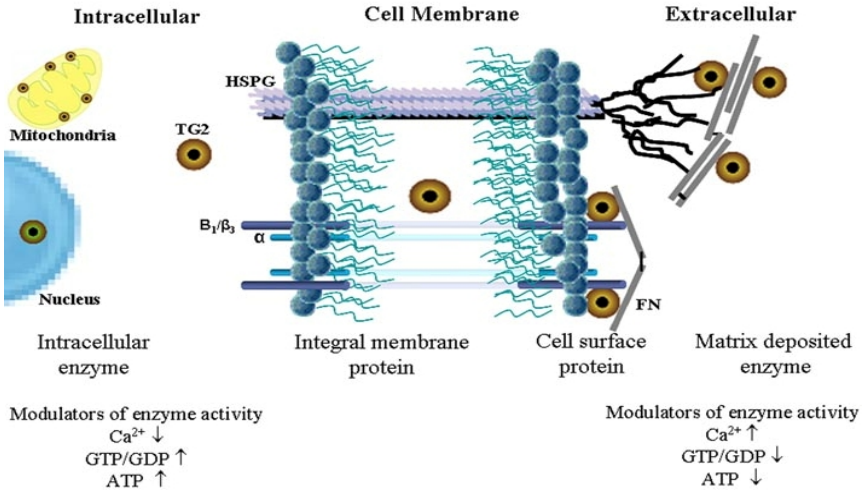

Fig. 2. Cellular distribution of TG2: The intracellular location of TG2 is predominantly in the cytosol though, the enzyme has been reported to be present in the nucleus and associated with the mitochondria. In addition, the figure shows a representation of the presence of TG2 in the membrane as it passes from the cytosol to the outside of the cell. At the cell surface TG2 may be associated with either beta1 or beta3 integrins in association with FN. The modulators of TG2 activity at the bottom of the figure refer to the activity state of TG2 when present inside the cell and when present at the cell surface and in the ECM as reviewed by Telci and Griffin [40].

The intracellular location of TG2 is predominantly in the cytosol, however the enzyme has also been reported to be present in the nucleus and associated with the mitochondria [40]. In essence, it is a cytosolic protein with greater proportion of its cellular pool (70-80%) present in the cytoplasm [5,41]. As a result of low concentration of Ca^{2+} within the cytoplasm, the transamidating activity of TG2 remains dormant inside the cell, while the protein functions as a GTPase [2,19]. However, cytosolic TG2 can be activated by most cellular stressors which trigger extracellular calcium ion influx or release of calcium ion from the intracellular stores [39]. Furthermore, the nuclear localization of TG2 has been reported to be approximately 5% or less [42]. Essentially, cytosolic TG2 migrates to the nucleus in response to specific stimuli

[43], and importin-3 is responsible for its translocation into the nucleus [44]; where it can either function as a G-protein [45] or as a transamidase activated by nuclear Ca^{2+} signals to cross-link histones [46].

A significant proportion of TG2 is found in association with membranes of different cell types [47]. The localization of TG2 on the surfaces of various cells types as well as in the extracellular matrix has been established [48]. Irrespective of the lack of a leader sequence or transmembrane domain, which would have helped in the translocation of TG2 to the surface by the conventional endoplasmic recticulum/golgi route, the enzyme is secreted from cells in a controlled manner [49, 50 and 51]. However, the mechanism of TG2 translocation across the phospholipid bilayer and the pathway of its externalization are not well understood. Available data have shown that the externalization of TG2 is determined by a number of factors, which include a fibronectin-binding site in the N-terminal β-sandwich domain of TG2 [50], the presence of a non-proline*cis* peptide bond at Tyr^{274} as justified by the loss of both the transamidation activity and secretion of the enzyme, following the mutation of this bond [52]. The third criterion for TG2 externalization is the presence of a Cys^{277} intact site, necessary for the deposition of the enzyme into the matrix [52]. Among these criteria, the presence of non-proline*cis* peptide bonds is a conserved feature in a number of transglutaminase [53], and was first identified in Factor XIII, which has two non-proline*cis* peptide bonds [54].

On externalization from the cell, cell surface TG2 has been shown to facilitate cellular interactions with the surrounding extracellular matrix (ECM); which are critical physiological processes underlying many key aspects of cell behavior, including cell adhesion, growth, migration, differentiation, programmed cell death, and ECM assembly [39]. In turn, these cellular processes are vital to embryogenesis and tissue morphogenesis, wound healing and tissue repair, as well as tumour growth and metastasis. In 1992, Gentile *et al* [55] first suggested the involvement of transglutaminase 2 in the mediation of cell-matrix adhesion. They observed an astonishing effect of TG2 over-expression on spreading of fibroblast and their increased resistance to trypsinization. Subsequent convincing proofs at both cellular and molecular levels have supported TG2 involvement in the mediation of cellular interactions with ECM; and demonstrated that TG2 serves as an adhesion receptor for fibronectin (FN) on the cell surface [56,57,58,59].

Fibronectin (FN) is a high molecular weight (~540kD) dimeric modular glycoprotein present in the plasma membrane and ECM [60]. It is synthesized by most cell types, where it interacts with a variety of adhesion receptors, including one or more fibronectin-binding integrins (α5β1, αVβ3, αVβ5, αVβ6, α4β1, α4β7, αIIbβ3, α8β1, α9β1), and other transmembrane proteins; resulting to cell proliferation, migration, and differentiation [61, 62]. Pathologically, FN is profoundly involved in wound healing, inflammation, blood clotting and thrombosis, as well as tumour growth and angiogenesis [39]. Fibronectin in its polymeric form, is represented in the extracellular matrix by fibrillar matrices [63], which do not only promote cell adhesion, but as well serve as a scaffold for assembly of other ECM molecules; and provide important orientations for surrounding cells, initiating cascades of signals upon interaction with cell surface receptors [64,65].

Transglutaminase 2 (TG2) has very high affinity for FN, to which it has been shown bind at 2:1 stoichiometry [66], independent of either Ca^{2+} or the transamidating and GTPase activities of TG2 [67]. The interaction of extracellular TG2 with FN has been shown to be involved in cell-matrix adhesion [57] and many other adhesion-dependent phenomena, such as cell migration, matrix assembly and signaling [68,69]. The gelatin-binding domain (42kD)

serves as the only binding site for TG2 on FN and binds TG2 with similar affinity as the whole FN [70]. Furthermore, the adhesive function of TG2 is favored by the fact that the 42kD gelatin-binding domain of FN contains no interaction sites for the numerous FN-binding integrins, as well as other FN-associated adhesion receptors [71]. Therefore, TG2 and integrins can independently bind distinct domains of FN, consequently existing in collaboration rather than engaging in competition in the cell adhesion process [39]. It has been established in different cell types that the binding of TG2 to the 42kD fragment of FN results to stable cell adhesion, limited spreading and formation of specialized adhesive structures at the cell-substrate interface [58, 68].

Regardless of the co-existence of TG2 and integrins at different FN-binding domains, where they streamline the cell adhesion process; TG2 also associates with integrins to maintain cell-extracellular matrix (ECM) interactions. Essentially, integrins represent a large class of transmembrane adhesion receptors constituted by non-related α and β subunits [72]. In all cell types apart from red blood cells, 24 integrin heterodimers constituted by 8 β subunits and 18 α subunits are expressed, serving as receptors for a number of ECM ligands and taking part in adhesion between cells [62,72]. The role of integrins in wound healing, blood clothing and thrombosis, viral and bacterial infection, inflammation, tumour growth and agiogenesis, as well as other pathological and physiological states; justified the fundamental functions of integrins in cell-matrix adhesion [39].

In different cell types, transglutaminase 2 has been shown to associate with many integrin receptors, by binding to the extracellular domains of the β1 and β3 integrin subunits [57, 58 and 68]. The stable non-covalent TG2-integrin complexes are formed independent of the transamidating activity of TG2, and there is no evidence of integrins serving as enzymatic substrates of TG2 or other transglutaminase [57]. Furthermore, Akimov*et al* [57] while performing a set of biochemical experiments performed on cells that do not synthesize FN demonstrated that the TG2-integrin interactionis not FN-mediated but direct. They further observed that integrin-TG2 complexes have 1:1 stoichiometry and all cell-surface TG2 is bound to integrin receptors; with the possibility of up to 40% of β1 integrin associating with TG2 in various cell types [57,68]. The ability of TG2 to form ternary adhesive complexes with integrins and FN, where all the three proteins successfully interact with each other [39]; highlights the importance of TG2 effects on cell adhesion and indicates an unconventional of TG2 as a co-receptor in cell-matrix interactions [57].

5. BIOCHEMISTRY OF TRANSGLUTAMINASE 2

Transglutaminase 2 is a multifunctional protein that serves as a mediator between several distinct biochemical functions at various cellular locations (Fig. 3). The diverse physiological implications of TG2 typify the correlations between its diverse biochemical activities and cellular functions.

Fundamentally, TG2 is part of the enzyme family responsible for the cross-linking of proteins and their consequent posttranslational modification through isopeptide bond formation between and within polypeptide chains [73]. These cross-linking activities of TG2 are Ca^{2+}-dependent and result from acyl-transfer reaction between γ-carboxamide group of a specific protein-bound glutamine and either the ε-amino group of a distinct protein-bound lysine residue or primary amines like polyamines and histamine [2]. The reaction primarily involves the exchange of primary amines for ammonia at the γ-carboxamide group of glutamine residues, in the presence of Ca^{2+} [73]. The binding of Ca^{2+} is vital to the cross-link formation because it initiates a conformational change that exposes a cysteine residue in the active

site domain; the cysteine reacts with the glutamine substrate, resulting to the formation of an acyl-enzyme intermediate and release of ammonia [74]. The subsequent reaction between the acyl-enzyme complex and a primary amine results to the formation of γ-glutamyl-amino cross-link, and concomitant release of the enzyme [74, 75 and 76]. The reaction mechanism of TG2 is schematically represented thus:

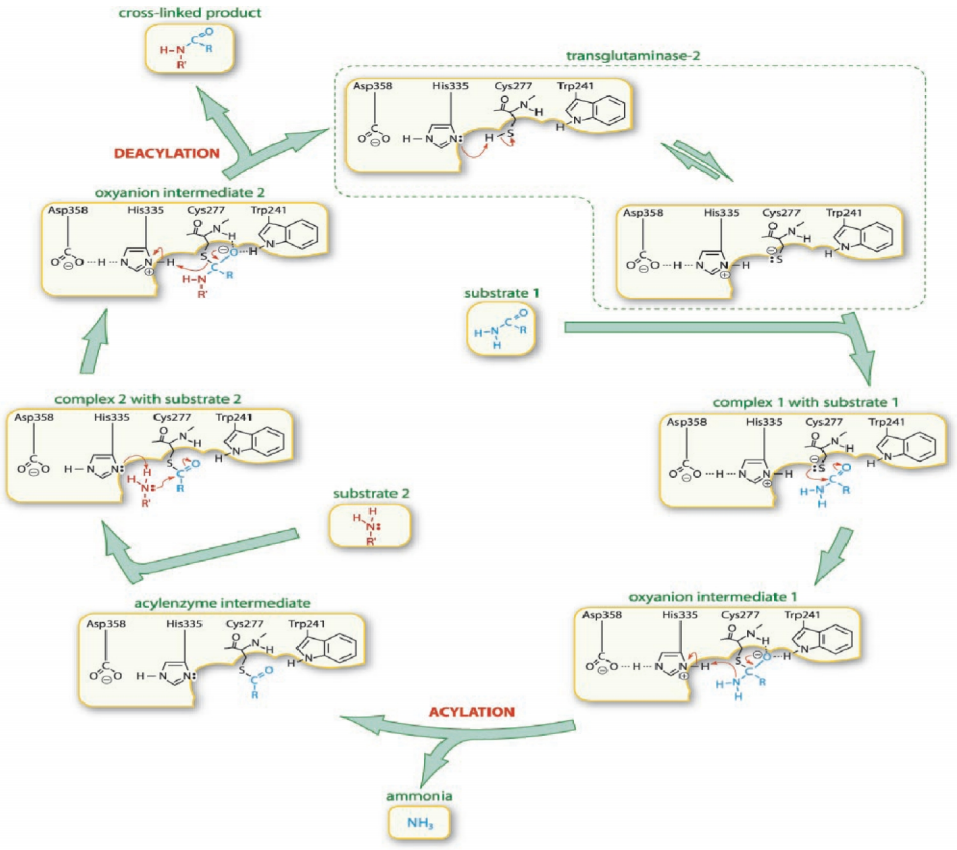

Fig. 3. Reaction pathways and proposed mechanism for TG2-catalysed transamidation, based on papain-reaction mechanism adapted from Iismaa et al. [74].

Other biochemical functions of TG2 include site-specific deamidation, during which water can replace amine donor substrate, amounting to the deamidation of the recognized glutamines [2]. Furthermore, TG2, just like factor XIIIA, exhibits isopeptidase activity in the presence of Ca^{2+}, and has been shown to hydrolyse γ: εisopeptides [77].

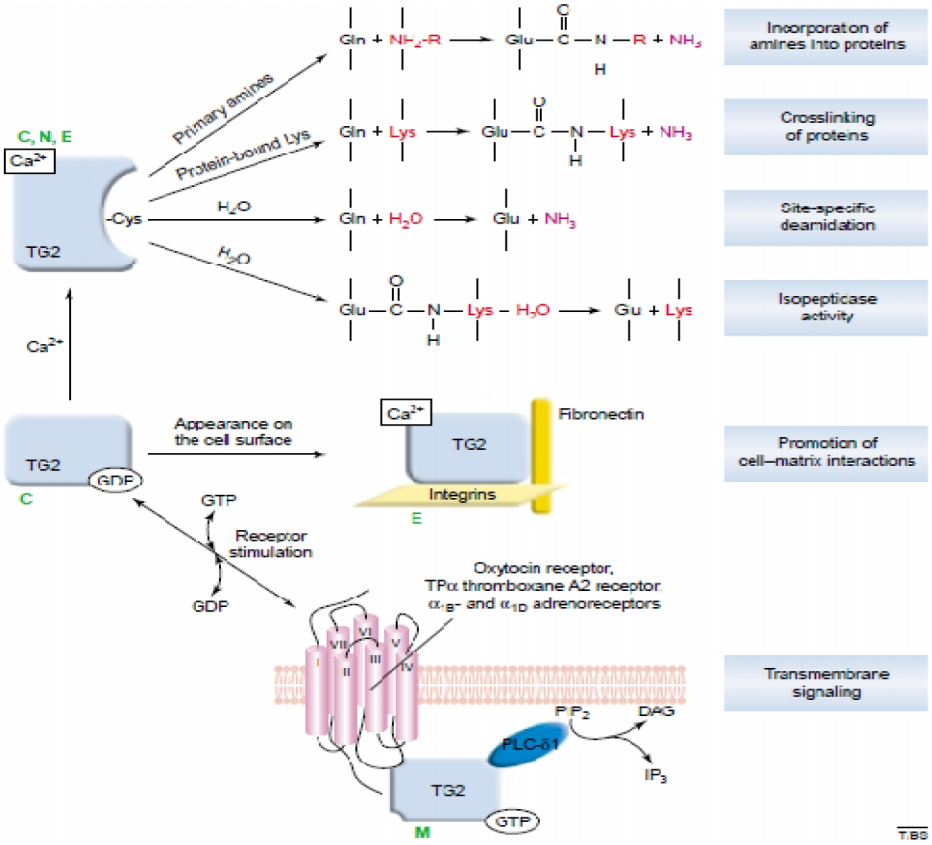

Fig. 4. Biochemical activities of TG2 at various cellular locations as reviewed by Fesus and Piacentini [2] and references therein. The cytosol, nucleus, cell membrane, and extracellular space are denoted with C, N, M, and E respectively.

At the cell membrane, TG2 plays a role in transmembrane signaling by transmitting signals from seven-transmembrane helix receptors to phospholipase C [34]. In essence, following the stimulation of these transmembranal helix receptors, TG2 binds and activate phospholipase C and proper regulation of the transmembrane signaling is ensured by its endogenous GTPase activity [78]. Transglutaminase 2 interaction with specific molecules such as sphingosylphosphocholine, could reduce the Ca^{2+} requirement for the transglutaminase activity [79]. This activity is highly influenced by nitric oxide such that up to 15 of the 18 cysteine residues can be nitrosylated and denitrosylated in a Ca^{2+} -dependent fashion, resulting to the enzyme inhibition and activation respectively [80].

A very striking part of TG2 function is its translocation to the nucleus under certain unknown conditions, with the help of importin-α3 [44]; where it could crosslink histone by nuclear Ca^{2+} -dependent activation, serving as a transamidase [46] or functioning as a G protein [45]. In a different vein, TG2 has been reported to be involved in the determination of the apoptotic fate of cells. Its over-expression primes cells for apoptosis and its inhibition by antisense strategy results to reduced cell death [81,82]. In 2002, Piacentini et al. [83] suggested that TG2 sensitizes cells for apoptosis by interacting with mitochondria, resulting to mitochondrial shift to higher polarized state and altered redox status; which might ignite the activation of

transglutaminase cross-linking activity [84]. During the later stage of apoptosis, membrane polarity is usually negated, resulting to a massive influx of Ca^{2+} into the cytosol. This increase in cytosolic Ca^{2+} leads to the acute activation of originally inactive TG2 to its cross-linking configuration in all sub-cellular compartments; and consequent extensive polymerization of intracellular proteins and formation of detergent-insoluble structures [85]. These insoluble protein scaffolds are functionally significant as they stabilize the structure of a dying cell prior to its phagocytotic clearance, hence, preventing the release of harmful intracellular components and the concomitant inflammatory or autoimmune responses [86].

6. REGULATION OF TG2 EXPRESSION AND CATALYTIC ACTIVITY

Transglutaminase 2 is involved in diverse physiological responses and as such, its expression is regulated by many factors. It can be regulated by various cytokines, hormones, and drugs [47,73,84]. The pattern of TG2 regulation has been demonstrated to be cell type-specific. For instance, the intracellular polyamines, spermine and spermidine, that serve as acyl acceptor substrates for transglutaminase [87,88] are capable of modulation of TG2 expression [73]. However, the blockage of polyamine synthesis in different cell types was shown to differently influence TG2 expression by effecting decreased expression in one cell type and increased expression in another [89,90].

Treatment of different cell types with natural and synthetic retinoids especially retinoic acid (RA), have been shown to induce dramatic increase in TG2 expression, at both transcriptional and translational (mRNA and protein) levels [91,92,93,94]. Retinoic acid-mediated induction of TG2 has also been demonstrated in vivo; where Verma et al. [95] observed a significant reduction in the level of TG2 in various tissues of a vitamin A-deficient rat, and increasing production of TG2 by the same tissues on resumption of vitamin A-containing diets.

From a catalytic perspective, transglutaminase 2 could be recognized as a bi-functional enzyme, owing to its ability to catalyze the Ca^{2+} -dependent protein cross-linking and Ca^{2+} -independent GTP and ATP hydrolysis [73]. In essence, the cross-linking function of TG2 is allosterically activated by Ca^{2+} -ion and reversibly inhibited by GTP, GDP, and GMP; whereas, it is not influenced by physiological concentrations of ATP or CTP [79, 95]. However, the GTPase and ATPase activity of TG2 occurs independent of Ca^{2+},but depends on Mg^{2+} -ions because Mg^{2+} -GTP and Mg^{2+} -ATP are the true substrates for TG2-mediated hydrolysis reaction [96]. In consonance with previous reports, Lai *et al* [96] demonstrated that the binding of Mg^{2+} -GTP complex to TG2 results to a conformational change which inhibits TG2 protein cross-linking activity without affecting its ATPase activity. They further established that the Mg^{2+} -ATP interaction with TG2 induces a conformational alteration that results in the inhibition of the GTPase activity without affecting its protein cross-linking propensity.In essence, these results suggest that the concentrations of Mg^{2+} -nucleotide complexes may be of vital importance in the modulation of TG2 activities. In a different vein, a membrane lipid, sphingosylphosphocholine (lyo-SM), has been suggested to be a potent activator of TG2 cross-linking activity [79].

The over-expression of TG2 does not necessarily lead to increased cross-linking activity. For instance, Smethurst and Griffin [97] while measuring TG2 activity in permeablized human endothelial cell system; showed that TG2 exists as a cryptic enzyme under physiological condition. This finding is particularly important as it demonstrated that the presence of TG2 is not always accompanied by its protein cross-linking activity inside living cells.

7. SUBSTRATE SPECIFICITY AND CELLULAR SUBSTRATE PROTEINS OF TG2

Transglutaminase 2 is a multifunctional protein with over 130 substrates at various locations inside and outside the cell [98]. This broad range of specificity of TG2 for its targets may account for its flexibility and multi-functionality. However, to achieve a particular function out of its variety of functions necessitates that the selection of specific subset of proteins related to that particular biological event must be tightly regulated by additional factors. Transglutaminase 2-specificity determining factors are numerous and include such factors as cell type- and tissue-dependent abundance of the enzyme and its substrate, availability of Ca^{2+}, the absence of inhibitors, the presence of modifying substances like sphyngosylphosphocholine [79] and nitric oxide [80], and the physical accessibility of modification sites on the individual molecules.

In essence, an understanding of the *in situ* TG2 substrates and its specificity to the substrates is needed in order to understand the physiological and pathological roles of TG2 [99]. The binding site in TG2 is organized in such a way that permits the proper orientation of peptide-bound residues of glutamine, whilst neither the free glutamine nor the asparagin is used by the enzyme; even in the midst of a strong stereo-specificity towards the L-isomer [100]. The possession of an extended active site by TG2 and its interaction with oligopeptides as proposed by Folk [100] influences the catalytic efficiency towards glutamine. The role of different amino acids in TG2 substrate effectiveness was studied by [101,102]. They observed that the positions of the different amino acids are important factors determining TG2 substrate requirement.

Transglutaminase 2 substrates are widely localised within the cellular and sub-cellular spaces of the cell. The recognition and post-translational modification of extracellular TG2 substrates have been implicated in some extracellular physiological functions like the stabilization of extracellular matrix (ECM) and cell-ECM interactions through the cross-linking of matrix proteins [47]. Fibronectin, an abundant extracellular protein, is a major TG2 substrate *in vitro* and *in vivo* [103]. Under normal cellular physiological conditions, TG2 externalized from cells becomes tightly bound to fibronectin and forms ternary complexes with collagens that function as a cementing substance in the ECM. This mechanism is used to clean up TG2 from the circulation, hence preventing it from causing any adverse effects [104]. Other TG2 substrates that are involved in the assembly, remodeling and stabilization of the ECM are fibrinogen/fibrin [105], von Willebrand factor [106], vitronectin [107], laminin and nidogen [75], liprotein(a) [108]. Transglutaminase 2 stabilises the reversible interactions between molecules that form heteromeric complexes in the ECM of specific tissues, e.g. laminin-nidogen [75], fibronectin-collagen [109], osteonectin-vitronectin [110].

Intracellularly, a large number of TG2 substrates abound, especially proteins involved in the organization of the cytoskeleton. As a result of its auto-catalytic activity, TG2 isoform in the cytoskeleton co-localises with stress fibers and cross-links myosin [104]. Upon activation by Ca^{2+}, TG2 contributes to the organization of the cytoskeleton by cross-linking various cytoskeletal proteins, such as β-tubulin, actin, microtubule protein tau, myosin, spectrin, thymosin β, troponin, and vimentin [111,112,113]. The function of this extensive polymerization which occurs at the final step of apoptosis is to stabilize the structures of the dying cells hence, preventing the release of cellular components that could cause inflammatory or autoimmune responses [2]. Furthermore, actin, retinoblastoma gene product, and nuclear proteins such as core histones, are TG2 substrates *in vivo* [114,115];

and the extensive polymerization of these proteins has been established as a key signal for the initiation of apoptosis [116]. In the subsequent section, the physiological implications of this wide range of TG2 specificity to various substrates in the cells will be discussed with pertinence to apoptosis, disease pathology, and cancer cell drug resistance and metastasis.

8. TRANSGLUTAMINASE 2 IN CELLULAR PHYSIOLOGY

Physiologically, the Ca^{2+}-dependent activation of TG2 has been implicated in many biological functions as diverse as extracellular matrix stabilization during development and wound healing, hormone receptor signal transduction as G-protein, cell growth and differentiation, cell adhesion and morphology, receptor-mediated endocytosis, cornified envelope formation in the keratinocytes, apoptosis, and cancer drug resistance and metastasis [73]. In this context, the roles of TG2 in apoptosis, disease pathology, and cancer drug resistance and metastasis will be reviewed.

8.1 Transglutaminase 2 in Apoptosis

In 1987, [81] on observing that lead-induced hypertrophy in the liver of rats was associated with an increased expression of TG2, suggested the initial link between TG2 and apoptosis. Since then, many reports have shown the involvement of TG2 in apoptosis [117] and references therein. Transglutaminase 2 involvement in apoptosis could be better described as a double-edged sword as it could be pro-apoptotic or anti-apoptotic. Essentially, cells undergoing apoptosis show an increased level of TG2 expression, which primes the cell to undergo apoptosis. Its inhibition however, results in a decreased propensity for cell death [118,119].

TG2-mediated pro-apoptosis is underlied by its cross-linking configuration, which requires a millimolar concentration of calcium. Stressful conditions such as ultraviolet radiation, chemotherapeutic agent, can generate reactive oxygen species (ROS) with resultant induction of TG2. Increase in the stressful conditions could further trigger the release of Ca^{2+} from the endoplasmic reticulum (ER), resulting to the activation of TG2 and extensive cross-linking of intracellular proteins, which in turn, initiates the apoptotic process [118, 120]. A major physiological significance of TG2 involvement in apoptotic initiation is its mediation of the crosstalk between dying and phagocytic cells to ensure tissue and cellular integrity. Essentially, the focal function of TG2 in apoptosis is to ensure that once the apoptotic process is initiated, it is completed without inflammation of tissue injury resulting from the process [121]. TG2 can achieve maintenance of a clean cellular environment whilst promoting apoptosis by directly promoting apoptosis in certain cell types [82, 122] or indirectly promoting the activation of TGF-β released by the macrophages that can promote the death of various cells [123,124], to ensure that all unwanted cells are killed fast without prior necrosis. Additionally, TG2 can promote chemo-attractant formation and the release of phosphatidylserine to respectively aid macrophage migration to the site of apoptosis and the recognition of apoptotic cells [121,125].

Just as TG2 could prime the cells to commit to death, so also could it protect the cells from dying. The anti-apoptotic effect of TG2 is mediated by TG2 in the nucleus and cell membrane. Nuclear TG2 protects cells from death by interacting with pRb, polymerising the alpha-inhibitory sub-unit of the transcription factor, NF-kappaB hence, regulating the transcription of several anti-apoptotic key genes [126]. Similarly, TG2 can translocate to the cell membranes where it serves as a co-receptor for integrins, promoting their interaction

with fibronectin. TG2-mediated interaction between integrins and fibronectin could result to the activation of cell survival and anti-apoptotic signaling pathways, and extracellular matrix stabilization [118]. Also, in the extracellular space, TG2 can resort to self-sustainability by activating latent transforming growth factor beta (TGF-β), which in turn up-regulates TG2 [121]. From the foregoing review of TG2 implication in apoptosis, it is tempting to conclude that the pro-apoptotic or anti-apoptotic effect of TG2 is dependent on the activation pathways and location; with nuclear and extracellular TG2 effecting anti-apoptosis while cytosolic TG2 is pro-apoptotic in consonance with the findings of [43].

8.2. Transglutaminase 2 in Disease Pathology

Owing to the multi-functionality and ubiquitous tissue distribution of TG2, it is not surprising that its involvement in many pathological conditions has been demonstrated. TG2 has been indicted for its roles in chronic diseases, especially in (a) inflammatory diseases, including wound healing, tissue repair and fibrosis, and autoimmune diseases; (b) chronic degenerative diseases such as arthritis, atherosclerosis, and neurodegenerative conditions like Alzheimer's and Parkinson disease; (c) malignant diseases; and (d) metabolic diseases such as diabetes mellitus [41,127]. In most of these diseases, the role of TG2 is mostly related to the deregulation of its functions especially, pertinent to its interaction with, and stabilization of cellular matrix, rather than its involvement in apoptosis.

In autoimmune diseases such as celiac disease, the presence of autoantibodies against TG2 and other substrates is an indication that TG2 may cross-link potential autoantigens to itself and to other protein substrates, to trigger an immunological response typical for autoimmune diseases [128,129]. TG2 function in celiac disease is related to the deamidation of the side chains of glutamine, which is abundant in gluten proteins. This diamidation reaction results to an improvement in the binding capacity of gluten to DQ2 and response of T-cell clones [130,131]. Additionally, it has been reported that gluten peptides incubated with TG2 create covalent complexes through thioester bond to active site cysteine of TG2 and via isopeptide bonds to particular lysine residues of TG2 [132]. Hence, gluten proteins and their peptide derivatives serve as substrates of various TG2-catalysed reactions [127]. In inflammatory diseases, TG2 plays its role via its regulatory action on granule secretion and macrophage function or by regulating the function of major inflammatory mediators like phospholipase A2 [133]. The involvement of TG2 in inflammatory diseases and related processes such as angiogenesis and wound healing has been reported [134,135]. It is an important player in the pathogenesis of chronic inflammatory diseases like rheumatoid arthritis and osteoarthritis by transforming the latent transforming growth factor binding protein-1 into its active form, TGF-β [136].

In vitro and/or *in vivo,* many TG2 substrates have been found in the neuron cellular compartments, e.g. amyloid beta-A4 peptide, alpha-synuclein, the microtubule-associated tau protein, synapsin I, and myelin basic protein [127] and references therein. TG2-mediated cross-linking is believed to be implicated in neurodegenerative diseases such as Huntington, Alzheimer and Parkinson diseases [129,137] and in diseases related to neurotransmitter release [138]. Similarly, the possible involvement of TG2 in neurotransmitter release and related pathological conditions like tetanus neurotoxin intoxication has been reported [139,140].

The covalent modification of TG2 substrates such as GAPDH, alpha-ketoglutarate dehydrogenase, phosphoglycerate dehydrogenase and fatty acid synthase [113] involved in energy metabolism, could account for the role of TG2 in metabolic diseases.

Additionally,TG2-mediated covalent modification of hormones receptors or hormone-binding proteins is an indication that TG2-catalysed cross-linking may be involved in controlling complex metabolic responses to hormones [141,142]. The involvement of TG2 in the regulation of insulin secretion, and diabetes mellitus has also been suggested [143,144].

Transglutaminase 2 is involved in the modulation of apoptosis and cell fate through many crucial cellular functions (as reviewed in the previous section). When aberrantly regulated, TG2 could aid cellular potentials to evade apoptosis. Evidently, there seems to be direct connection of TG2 with cancer drug resistance [145, 146] and mechanism of metastatic progression [147].

Many studies have demonstrated elevated TG2 expression as a hallmark of many types of cancer cells, including pancreatic carcinoma [148], ovarian carcinoma [149, 150], malignant melanoma [151], lung carcinoma [152], glioblastoma [153], and breast carcinoma [147]. For instance, [154] on analyzing the genes from tumour samples observed that out of over 30,000 genes analyzed, TG2 was among those that recoded the highest expression in pancreatic carcinoma. Similarly, [155], while attempting to identify metastasis-associated proteins through proteomic analysis, observed that TG2 was 1 of the 11 proteins that were constitutively elevated in metastatic human lung carcinoma. In another development, [156] showed that cancer cells treated with epidermal growth factor (EGF) expressed high level of TG2 and were consequently, protected cells from doxorubicin-induced apoptosis. These observations are strong reflectors of the implications of aberrant TG2 expression in the conferment of apoptotic resistance and consequent drug resistance and metastatic potentials of cancer cells. Similarly, metastatic tumors from patients with breast carcinoma [147], malignant melanoma [151], and ovarian carcinoma [149, 150] have been shown to express higher level TG2 relative to their primary counterparts. On the other hand, TG2 down-regulation or inhibition by small interfering RNA (siRNA), antisense RNA, ribozyme, or small molecule inhibitors have been shown to increase the susceptibility of various cancer cell types to chemotherapy-induced cell death, and to inhibit invasion, both *in vitro* and *in vivo* [119,149,150]. In their research, [149] observed that increased TG2 expression promoted the adhesion of ovarian cancer cells to fibronectin and facilitated directional cell migration, while TG2 down-regulation in similar cells decreasedtumour dissemination on the peritoneal surface and in mesentery in an intra-peritoneal ovarian xenograft mouse model. Put together, these observations strongly support that over-expression of TG2 confers resistance to chemotherapeutic drugs and promotes the invasive potential of malignant cells.

9. SUMMARY AND PERSPECTIVES

In this article, the history and biology of TG2 have been documented and many reports have shown that it is the most unique member of the family of transglutaminase enzymes. TG2 has demonstrated this uniqueness in many ways including specialized biochemical structure and ubiquitous tissue distribution, both of which allow for the flexibility of its interaction with myriads of proteins. This ability of TG2 to interact successfully with a wide range of proteins accounts for its multi-functionality, which in turn accounts for its correlation with many cellular functions.

Furthermore, regardless of the wide range of biological functionalities associated with TG2, amidst its unique cellular biochemistry, the exact physiological function remains unclear. This could be substantiated by the fact that homozygous deletion of TG2 in mouse does not result in an embryonic lethal phenotype [157,158]; suggesting a compensation for its absence by other family members. However, [143] observed that TG2- deficient mice

displayed characteristic glucose intolerance and hyperglycemia due to reduced insulin secretion, a condition equivalent to a subtype of diabetes called maturity-onset diabetes of the young (MODY). In humans, TG2-deficiency disease is yet to be identified.

Whilst there is existing evidence suggesting the possible compensation for the absence of TG2 by another member of the transglutaminase family, it is rational to think that the enzyme is actually involved in many physiological processes. This could be justified by the understanding that TG2 is relatively more abundant than other members of Tgase family. Consequently, its wide tissue distribution and the possession of specialized functional structure that allows for flexibility of interaction with assorted proteins are some of the factors that give TG2 a physiological advantage over other Tgasemembers. Additionally, it is appropriate to argue that the compensation for TG2 function could only be possible for a role that is determined by its cross-linking activity, which is a common feature of the transglutaminase family. For example, TG2-mediated functions that are independent of its cross-linking actions like its role as a G-protein, and regulation of energy metabolism, are seemingly impossible roles to be undertaken by any other member of the Tgase family. Similarly, TG2-mediated integrin-fibronectin interaction which is one of the major primary routes of extracellular survival signaling activation and consequent apoptotic evasion; is independent of TG2 cross-linking activity and could not be compensated for by another Tgase.

10. CONCLUSION

Here, we presented the history and biology of TG2; and available literature showed that it is the most unique member of the family of transglutaminase enzymes. TG2 has demonstrated this uniqueness in many ways including specialized biochemical structure and ubiquitous tissue distribution, both of which allow for the flexibility of its interaction with myriads of proteins. This ability of TG2 to interact successfully with a wide range of proteins accounts for its multi-functionality, which in turn accounts for its correlation with many cellular functions. The involvement of TG2 in many cellular physiologies and the growing interests of researchers in TG2-related research are the compelling factors for this paper. Hence, it is our hope that this article will be handy to both old and new interests in the field, helping them to understand the basics of this very important multifunctional enzyme.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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