Review Article

Biological Functionalities of Transglutaminase 2 and the Possibility of Its Compensation by Other Members of the Transglutaminase Family

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Transglutaminase 2 (TG2) is the most widely distributed and most abundantly expressed member of the transglutaminase family of enzymes, a group of intracellular and extracellular proteins that catalyze the Ca^{2+}-dependent posttranslational modification of proteins. It is a unique member of the transglutaminase family owing to its specialized biochemical, structural and functional elements, ubiquitous tissue distribution and subcellular localization, and substrate specificity. The broad substrate specificity of TG2 and its flexible interaction with numerous other gene products may account for its multiple biological functions. In addition to the classic Ca^{2+}-dependent transamidation of proteins, which is a hallmark of transglutaminase enzymes, additional Ca^{2+}-independent enzymatic and nonenzymatic activities of TG2 have been identified. Many such activities have been directly or indirectly implicated in diverse cellular physiological events, including cell growth and differentiation, cell adhesion and morphology, extracellular matrix stabilization, wound healing, cellular development, receptor-mediated endocytosis, apoptosis, and disease pathology. Given the wide range of activities of the transglutaminase gene family it has been suggested that, in the absence of active versions of TG2, its function could be compensated for by other members of the transglutaminase family. It is in the light of this assertion that we review, herein, TG2 activities and the possibilities and premises for compensation for its absence.

1. Introduction

The human transglutaminase 2 (TGM2) gene localizes to chromosome 20q11-12 and its exons span approximately 37 kb [1]. The protein, transglutaminase 2 (TG2, EC 2.3.2.13), is made up of 687 amino acids, with molecular mass of 77.3 kDa [2, 3]. Transglutaminase 2 (TG2) is also known as tissue transglutaminase (tTG), cytosolic, type II, or liver transglutaminase. It is the most abundant and most studied of the nine members of the transglutaminase enzyme family, including TG1, TG3, and TG5 isoforms, which are predominantly expressed in epithelial tissue; TG4, which is expressed in the prostate gland; factor XIII (FXIII), which is expressed in the blood; TG6 which is expressed in the testis, lungs, and brain [4, 5]; TG7, which is ubiquitously expressed but most prominently distributed in the testis and lungs [4].

A further member of the TG2 family is band 4.2, which is an enzymatically inactive protein component of the erythrocyte membrane that shares homology with many transglutaminases but lost the characteristic transglutaminase activity as a result of an amino acid substitution (Cys-Ala) at the active site [4, 6]. It is expressed in the surface of erythrocyte membranes, bone marrow, foetal liver, and spleen and serves as a key component of erythrocyte skeletal network, where it maintains erythrocyte shape and mechanical properties [4, 6] (Table 1).

Furthermore, shorter forms of TG2 with different properties have been reported to be produced through the alternative splicing of transglutaminase 2 encoding gene (TGM2) [3, 7–9]. A total of four spliced forms of TG2 have been reported, including TG2-S, TG2-H2, $TG2_{v1}$, and $TG2_{v2}$, but their roles *in vivo* are yet to be defined (as reviewed

TABLE 1: Members of the transglutaminase (TGase) enzyme family, their nomenclature, tissue distribution, biological functions, and pathological involvement [4, 30].

TGase	Nomenclature	Tissue distribution, cellular and subcellular localization	Biological functions	Pathology
TG1	Keratinocyte TG, particulate TG, TG1, and TGK	Squamous epithelia, keratinocytes, cytosolic, membrane	Barrier function in stratified squamous epithelia, cornified envelope formation in keratinocyte differentiation	Lamellar ichthyosis [119]
TG2	Liver TG, tissue TG, cytosolic TG, erythrocyte TG, Ghα, and endothelial TG	Ubiquitously distributed in many types of tissue, cell membrane, cytosol, nucleus, extracellular	Apoptosis, cell survival signalling, cell differentiation, matrix stabilization, endocytosis, and so forth	Autoimmune diseases, neurodegenerative diseases, malignancies, metabolic diseases, and so forth [78]
TG3	Epidermal TG, callus TG, hair follicle TG, and bovine snout TG	Epidermis, hair follicle, brain, cytosolic	Terminal differentiation of keratinocytes, hair follicles	Human epidermis diseases
TG4	Prostate TG, TGp, androgen regulated major secretory protein, vesiculase, dorsal prostate protein 1 (DP1), and type 4 TG	Prostate gland, prostatic fluids, and seminal plasma, extracellular	Reproduction and fertility, especially in rodents where it is involved in semen coagulation	Not known
TG5	TGX, type 5 TG, and TG5	Ubiquitously expressed but predominant in female reproductive tissues, skeletal muscle, and foetal tissues, foreskin keratinocytes, epithelial barrier lining, cytosolic	Epidermal differentiation	Secondarily involved in hyperkeratotic phenotype in ichthyosis and in psoriasis, overexpressed or absent in different areas of the Darier's disease lesions [120]
TG6	TGY, type 6 TG, and TG6,	Testis, lungs, and brain, cellular localization is yet to be defined	Central nervous system development, motor function, and late stage cell envelope formation in the epidermis and the hair follicle	Spinocerebellar ataxias [121, 122]; polyglutamine (polyQ) diseases [123]
TG7	TGZ, type 7 TG, TG7, and transglutaminase 7	Ubiquitously expressed, prominent in testis and lungs, cellular and subcellular localization are unknown		Not known
FXIIIA	Factor XIII A, fibrin stabilizing factor, fibrinoligase, plasma TG, and Laki-Lorand factor	Chondrocytes platelets, placenta, astrocytes, macrophages, synovial fluid, heart, eye, bone, dendritic cells in the dermis	Wound healing, blood clotting, and bone growth	F13A1 deficiency characterized by impaired wound healing, spontaneous abortion in women, subcutaneous and intramuscular haematomas, and severe bleeding tendency
Band 4.2	Erythrocyte membrane protein band 4.2, B4.2, ATP-binding erythrocyte membrane protein band 4.2	Surface of erythrocyte membranes, bone marrow, foetal liver, spleen, membranal	Key component of erythrocyte skeletal network maintains erythrocyte shape and mechanical properties	Spherocytic elliptocytosis

by [10]). The major difference between TG2 and its spliced isoforms is that all the spliced isoforms lose their C-terminus to different extents; therefore, they cannot undertake the characteristic functions of TG2 like GTP/ATP binding, PLC binding, migration and adhesion functions, and so forth [11–13]. Though, some of these isoforms of TG2 are yet to be properly characterised, TG2 short isoform (TG2-S), which lacks the C-terminal 138 amino acid residues of full length TG2, has been characterised and reported to be upregulated in brain tissue of Alzheimer's patients [14–16].

Structurally, TG2 is similar to those of other members of its family, except that it bears some specific features which are not characteristic of other type of transglutaminases. Essentially, TG2 is composed of four distinct globular domains: A NH_2-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 [17, 18], and two COOH-terminal β-barrel domains with the second barrel domain containing a phospholipase C binding sequence [19, 20].

Unlike other transglutaminases, TG2 possesses a unique guanidine nucleotide-binding site, located in the cleft between the catalytic core and the first β-barrel [20];

this sequence is coded by exon 10 of the TG2 gene, which is characterised by very poor sequence homology with the same exons in other transglutaminases. Some GDP/GTP-interacting residues and those necessary for GTP hydrolysis are situated in other domains [21]. 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 interdomain interaction between the catalytic domain 2 and domains 3 and 4, which reduces the accessibility of the active centre [20].

Besides the primary transglutaminase enzymes' activity of catalyzing the calcium-dependent posttranslational modification of proteins, TG2 can also bind and hydrolyze GTP [22], exhibit protein disulphide isomerase activity [23], and function as a protein kinase independently of calcium [24]. Furthermore, TG2 has calcium-independent nonenzymatic activities, especially extracellularly, where it interacts with a number of cell surface proteins [25], taking part in cell adhesion processes and stabilization of the extracellular matrix. This catalogue of special activities and multiple functionalities is underlined by TG2's structural uniqueness and complexity typified by its complex four-domain structure, constituted of an N-terminal β-sandwich, a catalytic core, and two C-terminal β-barrel domains [18]. Regardless of the wide range of biological functionalities associated with TG2 activities, amidst its unique cellular biochemistry, its exact physiological function is still debated. Hence, the view that in the event of its absence, the physiological function of TG2 could be compensated for by another member(s) of the transglutaminase family. It is against this background, that we considered the implications of TG2 expression and TG2-specific activities in biological processes. This is done with a view to rationalizing its involvement in many cellular physiological events and dissecting the premise that its functional compensation is feasible or unlikely.

2. Transglutaminase 2-Specific Enzymatic Activities

Catalysis of Ca^{2+}-dependent posttranslational modification of proteins is the major hallmark of transglutaminase enzymes. The mechanism of this reaction is generally the same for TG2 and other members of TG-enzyme family and involves a two-step process, as recently reviewed in Gundemir et al. [26]. The first step is the formation of a thioester bond with the enzyme's active cysteine site via the transamidation of the γ-carboxamide group of a peptide bond glutamyl substrate, which is accompanied by the release of ammonia as a by-product. This is followed by the transfer of the acyl intermediate to a nucleophilic substrate, including the ε-amino group of a peptide-bound lysine residue. Consequently, an intermolecular isopeptide ε-(γ-glutamyl)lysine bond is formed, which results in the internal cross-linking of monomeric protein units [27]. In transamidation reactions, lysine can be replaced by lower molecular mass amines, such as polyamines. Hence, in the presence of high concentration of polyamines, such as spermine, TG2 can form covalent

cross-linking between two polypeptide chains, in the form of a dimer or an adduct as in the case of Gln–Gln or Gln–Gly- [27]. These bonds are resistant to chemical and physical degradation; hence, they are believed to be of biological significance especially in the stabilization of the extracellular matrix (ECM) [25, 28]. In cellular physiology, the isopeptide bonds formed by TG2 activity have been suggested to be functionally important in apoptosis, where they prevent inflammation by ensuring that the intracellular contents of dying cells are not released to the extracellular environment [29]. However, water can also act as a nucleophile and cause deamidation of protein-bound glutamine residues [27, 30]. The conversion of the acyl-donor glutamine residue to a glutamate residue triggers deamination. Originally, it was believed that the deamination reaction could only take place under conditions that do not favour transamidation [26, 31]. However, studies have reported the tendency of TG2 to carry out specific deamination [32], when structural features that favour deamination over transamidation are present in protein substrates [33].

3. Calcium-Independent Nonenzymatic Activities of Transglutaminase 2

In addition to the general hallmark of calcium-dependent transamidation activity, TG2 has other enzymatic activities, independent of Ca^{2+} as reviewed in Gundemir et al. [26]. For instance, TG2 can function as a protein kinase [24, 34–37], bind and hydrolyze GTP (GTPase and G-protein function) [11, 19, 22, 38–42], and exhibit protein disulfide isomerase activity *in vitro* [23] and *in vivo* [43–45], independent of calcium as reviewed in Belkin [25].

Over the past two decades, other functions of TG2 that are independent of its enzymatic activities have been established [46–52]. These calcium-independent, nonenzymatic and transamidation-independent activities of TG2 are involved in many critical physiological processes underlying many key aspects of cell behaviour, including cell adhesion, growth, migration, differentiation, programmed cell death, and ECM assembly [49]. In turn, these cellular processes are vital to embryogenesis and tissue morphogenesis, wound healing, and tissue repair, as well as tumor growth and metastasis [53].

In 1992, Gentile and colleagues first suggested the involvement of transglutaminase 2 in the mediation of extracellular matrix (ECM) adhesion [54]. They observed an astonishing effect of TG2 overexpression on the growth of fibroblasts and their increased resistance to trypsinization. Subsequent convincing proofs, at both the cellular and molecular levels, have supported TG2's involvement in the mediation of cellular interactions with the ECM and have demonstrated that TG2 serves as an adhesion receptor for fibronectin (FN) on the cell surface [46, 55–57].

Transglutaminase 2 has a very high affinity for FN, to which it has been shown to bind at a stoichiometry of 2 : 1 [58], independently of either Ca^{2+} or the transamidating and GTPase activities of TG2 [59]. The interaction of TG2

with FN has been shown to mediate ECM adhesion [46] and many other adhesion-dependent phenomena, such as cell migration, matrix assembly, and signalling [60, 61]. The gelatin-binding domain (42 kD) serves as the only binding site for TG2 on FN and binds TG2 with similar affinity as the whole FN molecule [62]. Additionally, the adhesive function of TG2 is favored by the fact that the 42 kD gelatin-binding domain of FN has no interaction sites for the numerous FN-binding integrins, as well as other FN-associated adhesion receptors [63]. Therefore, TG2 and integrin can independently bind distinct domains of FN, hence supporting a model of cooperation rather than engaging in competition in the cell adhesion process [49]. It has been shown in different cell types that the binding of TG2 to the 42 kD fragment of FN results in stable cell adhesion, limited spreading, and formation of specialized adhesive structures at the cell-substrate interface [56, 60].

Regardless of the coexistence of TG2 and integrin at different FN-binding domains, where they are involved in the cell adhesion process, TG2 also associates with integrin to maintain cell-extracellular matrix (ECM) interactions. Integrins represent a large class of transmembrane adhesion receptors constituted by distinct noncovalently associated and are composed of α and β subunits [64]. In all cell types apart from red blood cells, 24 integrin heterodimers constituted variously of 8 β subunits and 18 α subunits are expressed, serving as receptors for a number of ECM ligands and facilitate adhesion between cells [64, 65]. The role of integrin in wound healing, blood clotting and thrombosis, viral and bacterial infection, inflammation, tumor growth, and angiogenesis, as well as other pathological and physiological states, exemplifies the fundamental functions of integrin in cell-matrix adhesion [49].

Transglutaminase 2 has been shown to be associated with many integrin receptors, by binding to the extracellular domains of the β1 and β3 integrin subunits in different cell types [46, 56, 60]. The stable, noncovalent TG2-integrin complexes are formed independently of the transamidating activity of TG2, and there is no evidence of integrin serving as an enzymatic substrate of TG2 or other transglutaminases [46]. Furthermore, while performing some biochemical experiments on cells that do not synthesize FN, Akimov et al. [46], demonstrated that the TG2-integrin interaction is not mediated by fibronectin but is independent. They further observed that integrin-TG2 complexes have 1 : 1 stoichiometry and found that cell-surface TG2 is bound to integrin receptors, with the possibility of up to 40% of β1 integrin being associated with TG2 in various cell types [46, 60]. The ability of TG2 to form ternary adhesive complexes with various isoforms of integrin and FN, where all the three proteins successfully interact with each other, highlights the importance of TG2 in cell adhesion and indicates an unconventional role of TG2 as a coreceptor in cell-matrix interactions [46]. The implications of these nonenzymatic, calcium- and transamidation-independent activities in some key biological events are highlighted below.

4. Implications of Transglutaminase 2 in Biological Events: A Synoptic Update

Transglutaminase 2 is a multifunctional protein with over 130 substrates at various locations inside and outside the cell [66] (Table 2). The broad range of specificity of TG2 for its targets may account for its pleiotropic functionality. However, to achieve a particular function necessitates that the selection of a specific subset of protein substrate related to that particular biological event must be tightly regulated by additional factors. The various physiological implications of TG2 typify the relationships between its diverse biochemical activities and cellular functions and make it difficult to determine the exact role it plays in cell physiology and pathology.

4.1. Transglutaminase 2 in Cell Survival and Death Processes. With regard to cell death and survival, the role of TG2 is extremely complex, and for almost three decades it has remained under investigation, following the first report of TG2's involvement in apoptosis [67]. Transglutaminase 2's involvement in apoptosis could be better described as a double-edged sword as it can be both proapoptotic or antiapoptotic. Cells undergoing apoptosis show an increased level of TG2 expression, which may prime the cell to undergo apoptosis. Its inhibition results in a decreased propensity of cells to die by apoptosis [68, 69].

4.1.1. Proapoptotic Activity of TG2. The proapoptotic activity of TG2 is defined by its cross-linking configuration, which requires a millimolar concentration of calcium. Stressful conditions, such as ultraviolet radiation and chemotherapeutic agents, can generate reactive oxygen species (ROS)—with resultant induction of TG2. Further increase in such stressful conditions may further trigger the release of Ca^{2+} from the endoplasmic reticulum (ER), resulting in the activation of TG2 and extensive cross-linking of intracellular proteins, which, in turn, initiates the apoptotic process [68, 70]. 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. In essence, the focal function of TG2 in apoptosis is to ensure that, once the apoptotic process is initiated, it is completed without inflammation or tissue injury resulting from the process [71]. TG2 can achieve maintenance of a cellular environment devoid of inflammation whilst directly promoting apoptosis in some cell types [72] or indirectly promoting the activation of TGF-β released by the macrophages, which can promote the death of various cells [73, 74], to ensure that all damaged cells are killed quickly without the occurrence of necrosis. Additionally, TG2 can promote chemoattractant formation and the release of phosphatidylserine, to, respectively, aid macrophage migration to the site of apoptosis and the recognition of apoptotic cells [71, 75].

4.1.2. Antiapoptotic Activity of TG2. The antiapoptotic effect of TG2 is independent of its transamidation and enzymatic cross-linking activities and does not require calcium. Nuclear

TABLE 2: Transglutaminase 2 substrates, reactive sites, cellular localizations, and possible involvement in human physiology/diseases.

TG2 substrate	Reactive site	Localization	Physiology/disease
Acetylcholine esterase	Glutamine	Intracellular	Neurological disease [53]
Actin	Glutamine and lysine	Intracellular	Cytoskeleton regulation [124]
Aldolase	Reactive glutamine present but specific residue is unknown	Intracellular	Genetic disease, endocrine and metabolic diseases, autoimmune and inflammatory diseases [125]
Androgen receptor		Intracellular (nuclear receptor)	Endocrine and metabolic diseases [97, 98]
Annexin I (lipocortin I)	Glutamine	Intracellular	Autoimmune and inflammatory diseases, cytoskeleton regulation [126]
Calgizzarin-S100C protein-MLN 70—S100A11	Glutamine and lysine	Keratinocyte cornified envelope	Endocrine and metabolic diseases, dermatological diseases [127]
Collagen alpha 1(III)	Glutamine	Extracellular	Extracellular matrix interaction and stabilization, autoimmune and inflammatory diseases [128]
α-B-crystallin	Lysine	Intracellular	Cell life and death, cytoskeleton regulation, protein stabilization [129]
β-A3 crystallin	Glutamine	Intracellular	Cell life and death, cytoskeleton regulation, protein stabilization [130]
β-B3 crystallin	Glutamine	Intracellular	Cell life and death, cytoskeleton regulation, protein stabilization [131]
β-Bp (beta-B2) crystalline	Glutamine	Intracellular	Cell life and death, cytoskeleton regulation, protein stabilization [131]
Cytochrome c	Glutamine	Intracellular	Cell life and death [132]
Fibronectin	Glutamine	Extracellular	Protein stabilization, extracellular matrix interaction and stabilization [68]
Fibrinogen A alpha	Glutamine and lysine	Extracellular	Extracellular matrix interaction and stabilization, autoimmune and inflammatory diseases [133]
Glutathione S-transferase	Glutamine, lysine, and fluorescein	Intracellular	Extracellular matrix interaction and stabilization [134]
Gluten proteins	Glutamine	Extracellular	Celiac disease [79]
Glyceraldehyde 3 phosphate dehydrogenase	Lysine	Intracellular	Neurological diseases [135]
H3 histone	Glutamine	Intracellular	Cell life and death [136]
H4 histone	Glutamine	Intracellular	Cell life and death [136]
H2A histone	Glutamine	Intracellular	Cell life and death [136]
H2B histone	Glutamine	Intracellular	Cell life and death [136]
Importin alpha 3		Nuclear transport protein	Cell life and death [34]
α-Ketoglutarate dehydrogenase	Lysine	Intracellular	endocrine and metabolic diseases [137]
Latent TGF-beta binding protein-1 (LTBP-1)		Extracellular	Carcinogenesis, autoimmune, and inflammatory diseases [138]
α-2-Macroglobulin receptor-associated protein	Glutamine	Extracellular	autoimmune and inflammatory diseases [139]
Microtubule-associated protein tau-isoform Tau-F (Tau-4)	Glutamine and lysine	Intracellular	Cytoskeleton regulation, neurological diseases [140]
Myosin		Intracellular	Cytoskeleton regulation [141]
Nidogen	Glutamine	Extracellular	Extracellular matrix interaction and stabilization [142]

TABLE 2: Continued.

TG2 substrate	Reactive site	Localization	Physiology/disease
Osteocalcin		Extracellular	Autoimmune and inflammatory diseases [143]
Osteonectin	Glutamine	Extracellular	Autoimmune and inflammatory diseases, extracellular matrix interaction, and stabilization [144]
Osteopontin	Glutamine	Extracellular	Autoimmune and inflammatory diseases, extracellular matrix interaction, and stabilization [145]
Phospholipase A2	Glutamine	Extracellular	Endocrine and metabolic diseases, signal transduction, autoimmune, and inflammatory diseases [2, 86]
Troponin T		Intracellular	Cytoskeleton regulation [146]

TG2 protect cells from death by interacting with retinoblastoma protein pRb, polymerizing the alpha-inhibitory subunit of the transcription factor NF-kappaβ, with the resultant transcriptional regulation of several antiapoptotic key genes [76]. Similarly, TG2 can translocate to the plasma membrane where it serves as a coreceptor for integrin, promoting its interaction with fibronectin. TG2-mediated interaction between integrin and fibronectin could result in the activation of cell survival and antiapoptotic signalling pathways, and extracellular matrix stabilization [68]. Also, in the extracellular space, TG2 can stimulate its own production by activating latent transforming growth factor beta (TGF-β), which in turn upregulates TG2 [71]. It is tempting to conclude that the proapoptotic and antiapoptotic effects of TG2 are dependent on the activation pathways and localization of the protein, with nuclear and extracellular TG2 as antiapoptotic and cytosolic TG2 is proapoptotic, in agreement with the findings of Milakovic et al. [77].

4.2. Transglutaminase 2 in Human Diseases. Owing to the pleiotropic and ubiquitous tissue distribution of TG2, it is not surprising that its involvement in many pathological conditions has been variously demonstrated. Transglutaminase 2 has been implicated as having a role in various 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's diseases; (c) malignant diseases; and (d) metabolic diseases such as diabetes mellitus [28, 78]. In most of these diseases, the role of TG2 is mostly related to the dysregulation of its functions, especially regarding its interaction with, and stabilization of the cellular matrix, rather than its involvement in apoptosis.

4.2.1. Transglutaminase 2 in Autoimmune Diseases. In autoimmune diseases such as coeliac 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, triggering an immunological response typical for autoimmune diseases [79, 80]. TG2's role in coeliac disease is related to the deamination of the side chains of glutamine, in the presence of abundant glutamine in gluten proteins. This deamination reaction results in an upregulation of the binding capacity of gluten to DQ2 and the response of T-cell clones [81, 82]. Additionally, it has been reported that gluten peptides incubated with TG2 create covalent complexes through thioester bonds to the active site cysteine of TG2 and via isopeptide bonds to particular lysine residues of TG2 [83]. Hence, gluten proteins and their peptide derivatives serve as substrates for various TG2-catalysed reactions [78]. Recently, deamidation of gluten-derived gliadin peptides by TG2 was shown to be responsible for gliadin-induced toxicity and immune response in the small-intestinal mucosa [84]. Consequently, Rauhavirta and colleagues suggested that the inhibition of TG2 can reduce gliadin-induced effects [84]. In a different study, Oh et al. [85] reported that the initiation of allergen response in pulmonary epithelial cells requires TG2.

4.2.2. Transglutaminase 2 in Inflammatory Diseases. In inflammatory diseases, TG2 plays a pivotal role via its regulatory action on granule secretion and macrophage function or by regulating the function of major inflammatory mediators like phospholipase A2 [86]. The involvement of TG2 in inflammatory diseases and related processes, such as angiogenesis and wound healing, has been reported [87, 88]. It is an important effector in the pathogenesis of chronic inflammatory diseases, like rheumatoid arthritis and osteoarthritis, by converting the latent transforming growth factor binding protein-1 into its active form, TGF-β [89]. Recently, TG2 has been reported to be directly involved in chronic kidney disease (CKD), where it is involved in the pathogenesis of vascular calcification through the enhancement of matrix vesicle-ECM interaction [90]. Similarly, on analysis of TG2 : creatinine ratio in relation to albumin : creatinine ratio in CKD patients, da Silva et al. [91] suggested that TG2 may be a potential biomarker for CKD detection and progression assessment.

4.2.3. Transglutaminase 2 in Neurological and Metabolic Diseases. In vitro and/or *in vivo*, many TG2 substrates have been found in neuronal cellular compartments, for example, amyloid beta-A4 peptide, alpha synuclein, the microtubule-associated tau protein, synapsin I, and myelin basic protein,

as reviewed by Facchiano et al. [78]. TG2-mediated cross-linking is therefore believed to be implicated in neurodegenerative diseases such as Huntington's, Alzheimer's, and Parkinson's diseases [79, 92] and in diseases related to neurotransmitter release [93]. Similarly, the possible involvement of TG2 in neurotransmitter release and related pathological conditions, such as that caused by tetanus neurotoxin intoxication, has been reported [94].

The covalent modification of TG2 substrates such as glyceraldehyde-3-phosphate dehydrogenase (GAPDH), alpha-ketoglutarate dehydrogenase, phosphoglycerate dehydrogenase, and fatty acid synthase [95], which are involved in energy metabolism, could account for the role of TG2 in metabolic diseases. Additionally, TG2-mediated covalent modification of hormone receptors or hormone-binding proteins indicates that TG2-catalysed cross-linking may be involved in controlling complex metabolic responses to hormones [96–98]. The involvement of TG2 in the regulation of insulin secretion and diabetes mellitus has also been suggested [99, 100].

4.2.4. Transglutaminase 2 in Cancer. In cancer, transglutaminase 2 has been shown to play a major role in development of drug resistance and metastasis in many cancer types, including pancreatic carcinoma [101], ovarian carcinoma [102, 103], malignant melanoma [104], lung carcinoma [105], glioblastoma [106], and breast carcinoma [107]. When aberrantly regulated, TG2 could aid tumor cells to evade apoptosis and have direct consequences on cancer drug resistance [108, 109] and metastatic progression [107]. For instance, Park et al. [110] reported that TG2-specific cross-linking activity resulted in the polymerization and inhibition of nucleophosmin and concomitant increase in drug resistance potential of cancer cells. Recent evidence shows that aberrant expression of TG2 in mammary epithelial cells confers stem cell characteristics on the cells [111]. Similarly, Kumar and colleagues reported that high basal expression of TG2 in breast cancer cells promotes the development of stem cell features but did not mediate their terminal differentiation [111]. Additionally, Caffarel et al. [112] observed that the activation of TG2: integrin-$\alpha 5\beta 1$ interactions through the stimulation of oncostatin M receptor in cervical squamous cell carcinoma induced promalignant changes.

Clinically, TG2 has been reported to serve as a predictive indicator of anticancer therapeutic efficacy. For instance, Jeong et al. [113] suggested that TG2 expression is a promising indicator of the effectiveness of epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) therapy in patients suffering from nonsmall cell lung cancer. Similarly, Jasmeet et al. [114] reported that the accumulation of TG2 in tumour stroma can serve as an independent risk factor for the identification of invasive ductal carcinomas (IDCs) of breast and can establish breast cancer patients at high risk of recurrence. They also observed that overexpression of TG2 can serve as an indicator of poor prognosis for IDC of the breast. Agnihotri et al. [115] proposed that inflammation-induced progression of breast cancer and acquisition of survival and invasive capabilities by breast cancer cells are mediated by

TG2. In acute myeloid leukaemia, Pierce et al. [116] demonstrated that increased expression of TG2 precipitated a more advanced state of the disease in relapse patients. They further established that increased TG2 expression correlates with the expression of proteins involved in apoptosis, motility, and extracellular matrix association and processes that have been linked with leukemia development and progression. This is a testament to the specialized ability of TG2 to interact with several proteins as substrates in various biological events, probably due to the unique biochemical structure of TG2 that is uncharacteristic of other transglutaminase enzymes.

5. Compensation for Transglutaminase 2 Functions

Regardless of the wide range of biological functionalities associated with TG2 and amidst its unique cellular biochemistry, its exact physiological functions are still debated. This debate is complicated by the fact that homozygous deletion of TG2 in mice does not result in an embryonic lethal phenotype [117, 118], suggesting that compensation for its absence may be achieved by other family members. Such knockouts are not however without associated pathology. For example, Bernassola et al. [100] observed that TG2-deficient mice displayed significant changes such as characteristic glucose intolerance and hyperglycaemia due to reduced insulin secretion, a condition equivalent to a subtype of diabetes called maturity-onset diabetes of the young (MODY). Moreover, a TG2-deficiency disease is yet to be identified in humans implying the importance of its presence.

This notion is further supported by the observation that TG2 is relatively more abundant than other members of the transglutaminase family. Its wide tissue distribution and its possession of a wide range of structural features that allows for flexibility in interaction with widely assorted proteins are some of the factors that give TG2 a potentially wide range of functions other than simple enzymatic activity.

Thus while there are increasing suggestions of possible compensation for the absence of TG2 by other members of the transglutaminase family [100], the pleiotropic nature of this protein indicates that TG2 is actually involved in more physiological processes than any other member of the enzyme family and, thus, while TG2 might feasibly compensate for other members of the family the suggestion that alternative TG isoforms may act as a universal "back up" system for TG2 seems less likely.

From the above, it seems more appropriate to argue that compensation for TG2's functions by other TGase isoforms might not be possible, except for roles that are determined by its calcium-dependent cross-linking and transamidating activities, which are common features of the transglutaminase family. For example, TG2-mediated functions that are enzymatic but independent of calcium, such as its role as a G-protein, protein disulphide isomerase activity, kinase function, and regulation of energy metabolism, are unlikely roles to be undertaken by any other member of the transglutaminase family. This could be due to absence of appropriate structural conformation in other transglutaminase enzymes

that could enable them to alternately assume such roles in the event of TG2 absence.

Similarly, TG2-mediated integrin-fibronectin interaction is critical to many physiological events in the cell, including cell adhesion, growth, migration, differentiation, programmed cell death, and ECM assembly [25, 49]. Such interaction is vital to many cellular processes and serves as one of the major routes for extracellular survival, signalling activation, and consequent apoptotic evasion. It is nonenzymatic and independent of TG2 transamidation and cross-linking activities. Consequently, it is unlikely that any other member of the transglutaminase family can successfully compensate for this function in the event of TG2 absence.

6. Conclusions

The abundance of TG2 in various cell types, its specialized structural conformation, and its broad substrate specificity are some of the key factors justifying the enzyme's implication in myriads of biological events. From this review, it is evident that besides its calcium-dependent activities, TG2 can enzymatically or nonenzymatically mediate key cell physiological events. However, it has been increasingly suggested that in the event of TG2 absence its biological functions could be successfully compensated for by other members of the transglutaminase family. These suggestions have been made without recourse to the distinguishing features of TG2 among the transglutaminase family. It is necessary to carry out further investigations to ascertain the main reasons why TG2 knockout is not embryonic lethal, instead of relying on the assertion that its functions are compensated for by other transglutaminase enzymes. Finally, it is also our view that a systematic investigation should be carried out to establish, with certainty, the possibility of and premise for the replacement of TG2 function by any other member of the transglutaminase family.

Conflict of Interests

There is no conflict of interests.

References

[1] V. Gentile, P. J. A. Davies, and A. Baldini, "The human tissue transglutaminase gene maps on chromosome 20q12 by in situ fluorescence hybridization," *Genomics*, vol. 20, no. 2, pp. 295–297, 1994.

[2] L. Fesus and M. Piacentini, "Transglutaminase 2: an enigmatic enzyme with diverse functions," *Trends in Biochemical Sciences*, vol. 27, no. 10, pp. 534–539, 2002.

[3] B. M. Fraij and R. A. Gonzales, "Organization and structure of the human tissue transglutaminase gene," *Biochimica et Biophysica Acta*, vol. 1354, no. 1, pp. 65–71, 1997.

[4] K. Mehta, "Mammalian transglutaminases: a family portrait," in *Transglutaminases: Family of Enzymes with Diverse Functions*, K. Mehta and R. Eckert, Eds., vol. 38 of *Progress in Experimental Tumor Research*, pp. 1–18, Karger Medical and Scientific Publishers, 2005.

[5] H. Thomas, K. Beck, M. Adamczyk et al., "Transglutaminase 6: a protein associated with central nervous system development

[6] L. Lorand and R. M. Graham, "Transglutaminases: crosslinking enzymes with pleiotropic functions," *Nature Reviews Molecular Cell Biology*, vol. 4, no. 2, pp. 140–156, 2003.

[7] B. M. Fraij, P. J. Birckbichler, M. K. Patterson Jr., K. N. Lee, and R. A. Gonzales, "A retinoic acid-inducible mRNA from human erythroleukemia cells encodes a novel tissue transglutaminase homologue," *The Journal of Biological Chemistry*, vol. 267, no. 31, pp. 22616–22623, 1992.

[8] B. M. Fraij, "GTP hydrolysis by human tissue transglutaminase homologue," *Biochemical and Biophysical Research Communications*, vol. 218, no. 1, pp. 45–49, 1996.

[9] B. M. Fraij and R. A. Gonzales, "A third human tissue transglutaminase homologue as a result of alternative gene transcripts," *Biochimica et Biophysica Acta*, vol. 1306, no. 1, pp. 63–74, 1996.

[10] T.-S. Lai and C. S. Greenberg, "TGM2 and implications for human disease: role of alternative splicing," *Frontiers in Bioscience*, vol. 18, pp. 504–519, 2013.

[11] M.-J. Im, M. A. Russell, and J.-F. Feng, "Transglutaminase II: a new class of GTP-Binding protein with new biological functions," *Cellular Signalling*, vol. 9, no. 7, pp. 477–482, 1997.

[12] S. N. P. Murthy, J. W. Lomasney, E. C. Mak, and L. Lorand, "Interactions of Gh/transglutaminase with phospholipase Cδ1 and with GTP," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 21, pp. 11815–11819, 1999.

[13] T. S. Lai, Y. Liu, W. Li, and C. S. Greenberg, "Identification of two GTP-independent alternatively spliced forms of tissue transglutaminase in human leukocytes, vascular smooth muscle, and endothelial cells," *FASEB Journal*, vol. 21, no. 14, pp. 4131–4143, 2007.

[14] B. A. Citron, K. S. SantaCruz, P. J. A. Davies, and B. W. Festoff, "Intron-exon swapping of transglutaminase mRNA and neuronal tau aggregation in Alzheimer's disease," *The Journal of Biological Chemistry*, vol. 276, no. 5, pp. 3295–3301, 2001.

[15] B. A. Citron, Z. Suo, K. SantaCruz, P. J. A. Davies, F. Qin, and B. W. Festoff, "Protein crosslinking, tissue transglutaminase, alternative splicing and neurodegeneration," *Neurochemistry International*, vol. 40, no. 1, pp. 69–78, 2002.

[16] M. A. Antonyak, J. M. Jansen, A. M. Miller, T. K. Ly, M. Endo, and R. A. Cerione, "Two isoforms of tissue transglutaminase mediate opposing cellular fates," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 49, pp. 18609–18614, 2006.

[17] S. N. P. Murthy, S. Iismaa, G. Begg, D. M. Freymann, R. M. Graham, and L. Lorand, "Conserved tryptophan in the core domain of transglutaminase is essential for catalytic activity," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 5, pp. 2738–2742, 2002.

[18] R. Király, M. Á. Demény, and L. Fésüs, "Protein transamidation by transglutaminase 2 in cells: a disputed Ca^{2+}-dependent action of a multifunctional protein," *FEBS Journal*, vol. 278, no. 24, pp. 4717–4739, 2011.

[19] K.-C. Hwang, C. D. Gray, N. Sivasubramanian, and M.-J. Im, "Interaction site of GTP binding G(h) (transglutaminase II) with phospholipase C," *The Journal of Biological Chemistry*, vol. 270, no. 45, pp. 27058–27062, 1995.

[20] S. Liu, R. A. Cerione, and J. Clardy, "Structural basis for the guanine nucleotide-binding activity of tissue transglutaminase and its regulation of transamidation activity," *Proceedings of*

and motor function," *Amino Acids*, vol. 44, no. 1, pp. 161–177, 2013.

the National Academy of Sciences of the United States of America, vol. 99, no. 5, pp. 2743–2747, 2002.

[21] S. E. Iismaa, M. Wu, N. Nanda, W. B. Church, and R. M. Graham, "GTP binding and signaling by G(h)/transglutaminase II involves distinct residues in a unique GTP-binding pocket," *The Journal of Biological Chemistry*, vol. 275, no. 24, pp. 18259–18265, 2000.

[22] H. Nakaoka, D. M. Perez, K. J. Baek et al., "G(h): a GTP-binding protein with transglutaminase activity and receptor signaling function," *Science*, vol. 264, no. 5165, pp. 1593–1596, 1994.

[23] G. Hasegawa, M. Suwa, Y. Ichikawa et al., "A novel function of tissue-type transglutaminase: protein disulphide isomerase," *Biochemical Journal*, vol. 373, no. 3, pp. 793–803, 2003.

[24] S. Mishra and L. J. Murphy, "Tissue transglutaminase has intrinsic kinase activity. Identification of transglutaminase 2 as an insulin-like growth factor-binding protein-3 kinase," *The Journal of Biological Chemistry*, vol. 279, no. 23, pp. 23863–23868, 2004.

[25] A. M. Belkin, "Extracellular TG2: emerging functions and regulation," *FEBS Journal*, vol. 278, no. 24, pp. 4704–4716, 2011.

[26] S. Gundemir, G. Colak, J. Tucholski, and G. V. W. Johnson, "Transglutaminase 2: a molecular Swiss army knife," *Biochimica et Biophysica Acta*, vol. 1823, no. 2, pp. 406–419, 2012.

[27] R. Porta, C. Esposito, S. Metafora et al., "Mass spectrometric identification of the amino donor and acceptor sites in a transglutaminase protein substrate secreted from rat seminal vesicles," *Biochemistry*, vol. 30, no. 12, pp. 3114–3120, 1991.

[28] M. Griffin, R. Casadio, and C. M. Bergamini, "Transglutaminases: nature's biological glues," *Biochemical Journal*, vol. 368, no. 2, pp. 377–396, 2002.

[29] B. Nicholas, P. Smethurst, E. Verderio, R. Jones, and M. Griffin, "Cross-linking of cellular proteins by tissue transglutaminase during necrotic cell death: a mechanism for maintaining tissue integrity," *Biochemical Journal*, vol. 371, no. 2, pp. 413–422, 2003.

[30] C. Esposito and I. Caputo, "Mammalian transglutaminases: identification of substrates as a key to physiological function and physiopathological relevance," *FEBS Journal*, vol. 272, no. 3, pp. 615–631, 2005.

[31] B. Fleckenstein, Ø. Molberg, S. Qiao et al., "Gliadin T cell epitope selection by tissue transglutaminase in celiac disease. Role of enzyme specificity and pH influence on the transamidation versus deamidation reactions," *The Journal of Biological Chemistry*, vol. 277, no. 37, pp. 34109–34116, 2002.

[32] J. Stamnaes, B. Fleckenstein, and L. M. Sollid, "The propensity for deamidation and transamidation of peptides by transglutaminase 2 is dependent on substrate affinity and reaction conditions," *Biochimica et Biophysica Acta*, vol. 1784, no. 11, pp. 1804–1811, 2008.

[33] D. M. Pinkas, P. Strop, A. T. Brunger, and C. Khosla, "Transglutaminase 2 undergoes a large conformational change upon activation," *PLoS Biology*, vol. 5, no. 12, article e327, 2007.

[34] T. Kuo, H. Tatsukawa, and S. Kojima, "New insights into the functions and localization of nuclear transglutaminase 2," *FEBS Journal*, vol. 278, no. 24, pp. 4756–4767, 2011.

[35] S. Mishra, A. Saleh, P. S. Espino, J. R. Davie, and L. J. Murphy, "Phosphorylation of histones by tissue transglutaminase," *The Journal of Biological Chemistry*, vol. 281, no. 9, pp. 5532–5538, 2006.

[36] S. Mishra and L. J. Murphy, "The p53 oncoprotein is a substrate for tissue transglutaminase kinase activity," *Biochemical and Biophysical Research Communications*, vol. 339, no. 2, pp. 726–730, 2006.

[37] S. Mishra, G. Melino, and L. J. Murphy, "Transglutaminase 2 kinase activity facilitates protein kinase A-induced phosphorylation of retinoblastoma protein," *The Journal of Biological Chemistry*, vol. 282, no. 25, pp. 18108–18115, 2007.

[38] K. E. Achyuthan and C. S. Greenberg, "Identification of a guanosine triphosphate-binding site on guinea pig liver transglutaminase. Role of GTP and calcium ions in modulating activity," *The Journal of Biological Chemistry*, vol. 262, no. 4, pp. 1901–1906, 1987.

[39] S. Chen, F. Lin, S. Iismaa, K. N. Lee, P. J. Birckbichler, and R. M. Graham, "α1-adrenergic receptor signaling via G(h) is subtype specific and independent of its transglutaminase activity," *The Journal of Biological Chemistry*, vol. 271, no. 50, pp. 32385–32391, 1996.

[40] J.-F. Feng, S. G. Rhee, and M.-J. Im, "Evidence that phospholipase δ1 is the effector in the G(h) (transglutaminase II)-mediated signaling," *The Journal of Biological Chemistry*, vol. 271, no. 28, pp. 16451–16454, 1996.

[41] K. J. Baek, N. S. Kwon, H. S. Lee, M. S. Kim, P. Muralidhar, and M.-J. Im, "Oxytocin receptor couples to the 80 kDa Ghx family protein in human myometrium," *Biochemical Journal*, vol. 315, no. 3, pp. 739–744, 1996.

[42] R. Vezza, A. Habib, and G. A. FitzGerald, "Differential signaling by the thromboxane receptor isoforms via the novel GTP-binding protein, G(h)," *The Journal of Biological Chemistry*, vol. 274, no. 18, pp. 12774–12779, 1999.

[43] P. G. Mastroberardino, M. G. Farrace, I. Viti et al., "'Tissue' transglutaminase contributes to the formation of disulphide bridges in proteins of mitochondrial respiratory complexes," *Biochimica et Biophysica Acta*, vol. 1757, no. 9-10, pp. 1357–1365, 2006.

[44] Z. Szondy, P. G. Mastroberardino, J. Váradi et al., "Tissue transglutaminase (TG2) protects cardiomyocytes against ischemia/reperfusion injury by regulating ATP synthesis," *Cell Death and Differentiation*, vol. 13, no. 10, pp. 1827–1829, 2006.

[45] W. Malorni, M. G. Farrace, P. Matarrese et al., "The adenine nucleotide translocator 1 acts as a type 2 transglutaminase substrate: implications for mitochondrial-dependent apoptosis," *Cell Death and Differentiation*, vol. 16, no. 11, pp. 1480–1492, 2009.

[46] S. S. Akimov, D. Krylov, L. F. Fleischmana, and A. M. Belkin, "Tissue transglutaminase is an integrin-binding adhesion coreceptor for fibronectin," *Journal of Cell Biology*, vol. 148, no. 4, pp. 825–838, 2000.

[47] R. Dardik and A. Inbal, "Complex formation between tissue transglutaminase II (tTG) and vascular endothelial growth factor receptor 2 (VEGFR-2): proposed mechanism for modulation of endothelial cell response to VEGF," *Experimental Cell Research*, vol. 312, no. 16, pp. 2973–2982, 2006.

[48] A. Janiak, E. A. Zemskov, and A. M. Belkin, "Cell surface transglutaminase promotes RhoA activation via integrin clustering and suppression of the Src-p190RhoGAP signaling pathway," *Molecular Biology of the Cell*, vol. 17, no. 4, pp. 1606–1619, 2006.

[49] E. A. Zemskov, A. Janiak, J. Hang, A. Waghray, and A. M. Belkin, "The role of tissue transglutaminase in cell-matrix interactions," *Frontiers in Bioscience*, vol. 11, no. 1, pp. 1057–1076, 2006.

[50] D. Telci, Z. Wang, X. Li et al., "Fibronectin-tissue transglutaminase matrix rescues RGD-impaired cell adhesion through syndecan-4 and β1 integrin co-signaling," *The Journal of Biological Chemistry*, vol. 283, no. 30, pp. 20937–20947, 2008.

[51] A. Scarpellini, R. Germack, H. Lortat-Jacob et al., "Heparan sulfate proteoglycans are receptors for the cell-surface trafficking

and biological activity of transglutaminase-2," *The Journal of Biological Chemistry*, vol. 284, no. 27, pp. 18411–18423, 2009.

[52] E. A. Zemskov, E. Loukinova, I. Mikhailenko, R. A. Coleman, D. K. Strickland, and A. M. Belkin, "Regulation of platelet-derived growth factor receptor function by integrin-associated cell surface transglutaminase," *The Journal of Biological Chemistry*, vol. 284, no. 24, pp. 16693–16703, 2009.

[53] D. Hand, D. Dias, and L. W. Haynes, "Stabilization of collagen-tailed acetylcholinesterase in muscle cells through extracellular anchorage by transglutaminase-catalyzed cross-linking," *Molecular and Cellular Biochemistry*, vol. 204, no. 1-2, pp. 65–76, 2000.

[54] V. Gentile, V. Thomazy, M. Piacentini, L. Fesus, and P. J. A. Davies, "Expression of tissue transglutaminase in Balb-C 3T3 fibroblasts: effects on cellular morphology and adhesion," *Journal of Cell Biology*, vol. 119, no. 2, pp. 463–474, 1992.

[55] E. Verderio, B. Nicholas, S. Gross, and M. Griffin, "Regulated expression of tissue transglutaminase in Swiss 3T3 fibroblasts: effects on the processing of fibronectin, cell attachment, and cell death," *Experimental Cell Research*, vol. 239, no. 1, pp. 119–138, 1998.

[56] A. M. Belkin, S. S. Akimov, L. S. Zaritskaya, B. I. Ratnikov, E. I. Deryugina, and A. Y. Strongin, "Matrix-dependent proteolysis of surface transglutaminase by membrane-type metalloproteinase regulates cancer cell adhesion and locomotion," *The Journal of Biological Chemistry*, vol. 276, no. 21, pp. 18415–18422, 2001.

[57] M. Kabir-Salmani, S. Shiokawa, Y. Akimoto, K. Sakai, K. Sakai, and M. Iwashita, "Tissue transglutaminase at embryo-maternal interface," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 8, pp. 4694–4702, 2005.

[58] E. K. LeMosy, H. P. Erickson, W. F. Beyer et al., "Visualization of purified fibronectin transglutaminase macrophage and human endothelial cell tissue transglutaminase," *The Journal of Biological Chemistry*, vol. 266, pp. 478–483, 1992.

[59] P. M. Turner and L. Lorand, "Complexation of fibronectin with tissue transglutaminase," *Biochemistry*, vol. 28, no. 2, pp. 628–635, 1989.

[60] S. S. Akimov and A. M. Belkin, "Cell-surface transglutaminase promotes fibronectin assembly via interaction with the gelatin-binding domain of fibronectin: a role in TGF-β-dependent matrix deposition," *Journal of Cell Science*, vol. 114, no. 16, pp. 2989–3000, 2001.

[61] E. A. M. Verderio, D. Telci, A. Okoye, G. Melino, and M. Griffin, "A novel RGD-independent cell adhesion pathway mediated by fibronectin-bound tissue transglutaminase rescues cells from anoikis," *The Journal of Biological Chemistry*, vol. 278, no. 43, pp. 42604–42614, 2003.

[62] J. T. Radek, J. Jeong, S. N. P. Murthy, K. C. Ingham, and L. Lorand, "Affinity of human erythrocyte transglutaminase for a 42-kDa gelatin-binding fragment of human plasma fibronectin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 8, pp. 3152–3156, 1993.

[63] J. Hang, E. A. Zemskov, L. Lorand, and A. M. Belkin, "Identification of a novel recognition sequence for fibronectin within the NH2-terminal β-sandwich domain of tissue transglutaminase," *The Journal of Biological Chemistry*, vol. 280, no. 25, pp. 23675–23683, 2005.

[64] R. O. Hynes, "Integrins: bidirectional, allosteric signaling machines," *Cell*, vol. 110, no. 6, pp. 673–687, 2002.

[65] M. J. Humphries, M. A. Travis, K. Clark, and A. P. Mould, "Mechanisms of integration of cells and extracellular matrices by integrins," *Biochemical Society Transactions*, vol. 32, no. 5, pp. 822–825, 2004.

[66] É. Csosz, B. Meskó, and L. Fésüs, "Transdab wiki: the interactive transglutaminase substrate database on web 2.0 surface," *Amino Acids*, vol. 36, no. 4, pp. 615–617, 2009.

[67] L. Fesus, V. Thomazy, and A. Falus, "Induction and activation of tiussue transglutaminase during programmed cell death," *The FEBS Letters*, vol. 224, no. 1, pp. 104–108, 1987.

[68] K. Mehta, J. Y. Fok, and L. S. Mangala, "Tissue transglutaminase: from biological glue to cell survival cues," *Frontiers in Bioscience*, vol. 11, no. 1, pp. 173–185, 2006.

[69] A. Verma and K. Mehta, "Tissue transglutaminase-mediated chemoresistance in cancer cells," *Drug Resistance Updates*, vol. 10, no. 4-5, pp. 144–151, 2007.

[70] L. S. Mangala and K. Mehta, "Tissue transglutaminase (TG2) in cancer biology," *Progress in Experimental Tumor Research*, vol. 38, pp. 125–138, 2005.

[71] L. Fésüs and Z. Szondy, "Transglutaminase 2 in the balance of cell death and survival," *The FEBS Letters*, vol. 579, no. 15, pp. 3297–3302, 2005.

[72] S. Oliverio, A. Amendola, C. Rodolfo, A. Spinedi, and M. Piacentini, "Inhibition of "tissue" transglutaminase increases cell survival by preventing apoptosis," *The Journal of Biological Chemistry*, vol. 274, no. 48, pp. 34123–34128, 1999.

[73] Z. Szondy, Z. Sarang, P. Molnár et al., "Transglutaminase 2-/- mice reveal a phagocytosis-associated crosstalk between macrophages and apoptotic cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 13, pp. 7812–7817, 2003.

[74] X. Huang and C. Lee, "From TGF-β to cancer therapy," *Current Drug Targets*, vol. 4, no. 3, pp. 243–250, 2003.

[75] H. Nishiura, Y. Shibuya, and T. Yamamoto, "S19 ribosomal protein cross-linked dimer causes monocyte-predominant infiltration by means of molecular mimicry to complement C5a," *Laboratory Investigation*, vol. 78, no. 12, pp. 1615–1623, 1998.

[76] J. E. Boehm, U. Singh, C. Combs, M. A. Antonyak, and R. A. Cerione, "Tissue transglutaminase protects against apoptosis by modifying the tumor suppressor protein p110 Rb," *The Journal of Biological Chemistry*, vol. 277, no. 23, pp. 20127–20130, 2002.

[77] T. Milakovic, J. Tucholski, E. McCoy, and G. V. W. Johnson, "Intracellular localization and activity state of tissue transglutaminase differentially impacts cell death," *The Journal of Biological Chemistry*, vol. 279, no. 10, pp. 8715–8722, 2004.

[78] F. Facchiano, A. Facchiano, and A. M. Facchiano, "The role of transglutaminase-2 and its substrates in human diseases," *Frontiers in Bioscience*, vol. 11, no. 2, pp. 1758–1773, 2006.

[79] S. Y. Kim, T. M. Jeitner, and P. M. Steinert, "Transglutaminases in disease," *Neurochemistry International*, vol. 40, no. 1, pp. 85–103, 2002.

[80] L. M. Sollid, O. Molberg, S. Mcadam, and K. E. A. Lundin, "Autoantibodies in coeliac disease: tissue transglutaminase guilt by association?" *Gut*, vol. 41, no. 6, pp. 851–852, 1997.

[81] H. Quarsten, O. Molberg, L. Fugger, S. N. McAdam, and L. M. Sollid, "HLA binding and T cell recognition of a tissue transglutaminase-modified gliadin epitope," *European Journal of Immunology*, vol. 29, no. 8, pp. 2506–2514, 1999.

[82] H. Arentz-Hansen, R. Körner, Ø. Molberg et al., "The intestinal T cell response to α-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase," *Journal of Experimental Medicine*, vol. 191, no. 4, pp. 603–612, 2000.

[83] B. Fleckenstein, S. Qiao, M. R. Larsen, G. Jung, P. Roepstorff, and L. M. Sollid, "Molecular characterization of covalent complexes between tissue transglutaminase and gliadin peptides," *The Journal of Biological Chemistry*, vol. 279, no. 17, pp. 17607–17616, 2004.

[84] T. Rauhavirta, M. Oittinen, R. Kivistö et al., "Are transglutaminase 2 inhibitors able to reduce gliadin-induced toxicity related to celiac disease? A proof-of-concept study," *Journal of Clinical Immunology*, vol. 33, no. 1, pp. 134–142, 2013.

[85] K. Oh, M. W. Seo, G. Y. Lee et al., "Airway epithelial cells initiate the allergen response through transglutaminase 2 by inducing IL-33 expression and a subsequent Th2 response," *Respiratory Research*, vol. 14, article 35, 2013.

[86] E. Cordella-Miele, L. Miele, and A. B. Mukherjee, "A novel transglutaminase-mediated post-translational modification of phospholipase A2 dramatically increases its catalytic activity," *The Journal of Biological Chemistry*, vol. 265, no. 28, pp. 17180–17188, 1990.

[87] J. Sohn, T. Kim, Y. Yoon, J. Kim, and S. Kim, "Novel transglutaminase inhibitors reverse the inflammation of allergic conjunctivitis," *Journal of Clinical Investigation*, vol. 111, no. 1, pp. 121–128, 2003.

[88] E. A. M. Verderio, T. S. Johnson, and M. Griffin, "Transglutaminases in wound healing and inflammation," *Progress in Experimental Tumor Research*, vol. 38, pp. 89–114, 2005.

[89] I. Nunes, P. Gleizes, C. N. Metz, and D. B. Rifkin, "Latent transforming growth factor-β binding protein domains involved in activation and transglutaminase-dependent cross-linking of latent transforming growth factor-β," *Journal of Cell Biology*, vol. 136, no. 5, pp. 1151–1163, 1997.

[90] N. X. Chen, K. O'Neill, X. Chen, K. Kiattisunthorn, V. H. Gattone, and S. M. Moe, "Transglutaminase 2 accelerates vascular calcification in chronic kidney disease," *The American Journal of Nephrology*, vol. 37, pp. 191–198, 2013.

[91] M. L. da Silva, M. El Nahas, and T. S. Johnson, "Urinary transglutaminase 2 as a potential biomarker of chronic kidney disease detection and progression," *The Lancet*, vol. 381, supplement 1, article S33, 2013.

[92] C. D. Bailey, J. Tucholski, and G. V. W. Johnson, "Transglutaminases in neurodegenerative disorders," *Progress in Experimental Tumor Research*, vol. 38, pp. 139–157, 2005.

[93] F. Deloye, F. Doussau, and B. Poulain, "Action mechanisms of botulinum neurotoxins and tetanus neurotoxins," *Comptes Rendus des Séances de la Société de Biologie et de ses Filiales*, vol. 191, no. 3, pp. 433–450, 1997.

[94] F. Facchiano and A. Luini, "Tetanus toxin potently stimulates tissue transglutaminase. A possible mechanism of neurotoxicity," *The Journal of Biological Chemistry*, vol. 267, no. 19, pp. 13267–13271, 1992.

[95] S. Orru, I. Caputo, A. D'Amato, M. Ruoppololl, and C. Esposito, "Proteomics identification of acyl-acceptor and acyl-donor substrates for transglutaminase in a human intestinal epithelial cell line. Implications for celiac disease," *The Journal of Biological Chemistry*, vol. 278, no. 34, pp. 31766–31773, 2003.

[96] K. Sakai, W. H. Busby Jr., J. B. Clarke, and D. R. Clemmons, "Tissue transglutaminase facilitates the polymerization of insulin-like growth factor-binding protein-1 (IGFBP-1) and leads to loss of IGFBP-1's ability to inhibit insulin-like growth factor-I-stimulated protein synthesis," *The Journal of Biological Chemistry*, vol. 276, no. 12, pp. 8740–8745, 2001.

[97] L. M. Mandrusiak, L. K. Beitel, X. Wang et al., "Transglutaminase potentiates ligand-dependent proteasome dysfunction induced by polyglutamine-expanded androgen receptor," *Human Molecular Genetics*, vol. 12, no. 13, pp. 1497–1506, 2003.

[98] L. M. Mandrusiak, L. K. Beitel, X. Wang et al., "Transglutaminase potentiates ligand-dependent proteasome dysfunction induced by polyglutamine-expanded androgen receptor," *Human Molecular Genetics*, vol. 12, no. 13, pp. 1497–1506, 2003.

[99] P. J. Bungay, J. M. Potter, and M. Griffin, "The inhibition of glucose-stimulated insulin secretion by primary amines. A role for transglutaminase in the secretory mechanism," *Biochemical Journal*, vol. 219, no. 3, pp. 819–827, 1984.

[100] F. Bernassola, M. Federiciz, M. Corazzari et al., "Role of transglutaminase 2 in glucose tolerance: knockout mice studies and a putative mutation in a MODY patient," *FASEB Journal*, vol. 16, no. 11, pp. 1371–1378, 2002.

[101] A. Verma, H. Wang, B. Manavathi et al., "Increased expression of tissue transglutaminase in pancreatic ductal adenocarcinoma and its implications in drug resistance and metastasis," *Cancer Research*, vol. 66, no. 21, pp. 10525–10533, 2006.

[102] M. Satpathy, L. Cao, R. Pincheira et al., "Enhanced peritoneal ovarian tumor dissemination by tissue transglutaminase," *Cancer Research*, vol. 67, no. 15, pp. 7194–7202, 2007.

[103] J. Y. Hwang, L. S. Mangala, J. Y. Fok et al., "Clinical and biological significance of tissue transglutaminase in ovarian carcinoma," *Cancer Research*, vol. 68, no. 14, pp. 5849–5858, 2008.

[104] J. Y. Kok, S. Ekmekcioglu, and K. Mehta, "Implications of tissue transglutaminase expression in malignant melanoma," *Molecular Cancer Therapeutics*, vol. 5, no. 6, pp. 1493–1503, 2006.

[105] K. S. Park, H. Kim, J. Lee et al., "Transglutaminase 2 as a cisplatin resistance marker in non-small cell lung cancer," *Journal of Cancer Research and Clinical Oncology*, vol. 136, no. 4, pp. 493–502, 2010.

[106] L. Yuan, M. Siegel, K. Choi et al., "Transglutaminase 2 inhibitor, KCC009, disrupts fibronectin assembly in the extracellular matrix and sensitizes orthotopic glioblastomas to chemotherapy," *Oncogene*, vol. 26, no. 18, pp. 2563–2573, 2007.

[107] K. Mehta, J. Fok, F. R. Miller, D. Koul, and A. A. Sahin, "Prognostic significance of tissue transglutaminase in drug resistant and metastatic breast cancer," *Clinical Cancer Research*, vol. 10, no. 23, pp. 8068–8076, 2004.

[108] K. Mehta, "High levels of transglutaminase expression in doxorubicin-resistant human breast carcinoma cells," *International Journal of Cancer*, vol. 58, no. 3, pp. 400–406, 1994.

[109] J. S. K. Chen, N. Agarwal, and K. Mehta, "Multidrug-resistant MCF-7 breast cancer cells contain deficient intracellular calcium pools," *Breast Cancer Research and Treatment*, vol. 71, no. 3, pp. 237–247, 2002.

[110] K. Park, B. Han, K. H. Lee et al., "Depletion of nucleophosmin via transglutaminase 2 cross-linking increases drug resistance in cancer cells," *Cancer Letters*, vol. 274, no. 2, pp. 201–207, 2009.

[111] A. Kumar, H. Gao, J. Xu, J. Reuben, D. Yu, and K. Mehta, "Evidence that aberrant expression of tissue transglutaminase promotes stem cell characteristics in mammary epithelial cells," *PLoS ONE*, vol. 6, no. 6, Article ID e20701, 2011.

[112] M. M. Caffarel, A. Chattopadhyay, A. M. Araujo, J. Bauer, C. G. Scarpini, and N. Coleman, "Tissue transglutaminase mediates the pro-malignant effects of oncostatin M receptor over-expression in cervical squamous cell carcinoma," *Journal of Pathology*, vol. 231, no. 2, pp. 168–179, 2013.

[113] J. H. Jeong, B. C. Cho, H. S. Shim et al., "Transglutaminase 2 expression predicts progression free survival in non-small cell lung cancer patients treated with epidermal growth factor receptor tyrosine kinase inhibitor," *Journal of Korean Medical Science*, vol. 28, no. 7, pp. 1005–1014, 2013.

[114] A. Jasmeet, G. Srivastava, A. Matta, M. C. Chang, P. G. Walfish, and R. Ralhan, "Transglutaminase 2 overexpression in tumor stroma identifies invasive ductal carcinomas of breast at high risk of recurrence," *PLoS ONE*, vol. 8, no. 9, Article ID e74437, 2013.

[115] N. Agnihotri, S. Kumar, and K. Mehta, "Tissue transglutaminase as a central mediator in inflammatory-induced progression of breast cancer," *Breast Cancer Research*, vol. 15, article 202, 2013.

[116] A. Pierce, A. D. Whetton, S. Meyer et al., "Transglutaminase 2 expression in acute myeloid leukemia: association with adhesion molecule expression and leukemic blast motility," *Proteomics*, vol. 13, no. 14, pp. 2216–2224, 2013.

[117] V. de Laurenzi and G. Melino, "Gene disruption of tissue transglutaminase," *Molecular and Cellular Biology*, vol. 21, no. 1, pp. 148–155, 2001.

[118] N. Nanda, S. E. Iismaa, W. A. Owens, A. Husain, F. Mackay, and R. M. Graham, "Targeted inactivation of Gh/tissue transglutaminase II," *The Journal of Biological Chemistry*, vol. 276, no. 23, pp. 20673–20678, 2001.

[119] E. Candi, G. Melino, A. Lahm et al., "Transglutaminase 1 mutations in lamellar ichthyosis: loss of activity due to failure of activation by proteolytic processing," *The Journal of Biological Chemistry*, vol. 273, no. 22, pp. 13693–13702, 1998.

[120] E. Candi, S. Oddi, A. Paradisi et al., "Expression of transglutaminase 5 in normal and pathologic human epidermis," *Journal of Investigative Dermatology*, vol. 119, no. 3, pp. 670–677, 2002.

[121] J. L. Wang, X. Yang, K. Xia et al., "TGM6 identified as a novel causative gene of spinocerebellar ataxias using exome sequencing," *Brain*, vol. 133, part 12, pp. 3510–3518, 2010.

[122] A. Sailer and H. Houlden, "Recent advances in the genetics of cerebellar ataxias," *Current Neurology and Neuroscience Reports*, vol. 12, no. 3, pp. 227–236, 2012.

[123] W. J. Guan, K. D. Xia, Y. T. Ma et al., "Transglutaminase 6 interacts with polyQ proteins and promotes the formation of polyQ aggregates," *Biochemical and Biophysical Research Communications*, vol. 437, no. 1, pp. 94–100, 2013.

[124] Z. Nemes Jr., R. Adány, M. Balázs, P. Boross, and L. Fésüs, "Identification of cytoplasmic actin as an abundant glutaminyl substrate for tissue transglutaminase in HL-60 and U937 cells undergoing apoptosis," *The Journal of Biological Chemistry*, vol. 272, no. 33, pp. 20577–20583, 1997.

[125] K. N. Lee, M. D. Maxwell, M. K. Patterson Jr., P. J. Birckbichler, and E. Conway, "Identification of transglutaminase substrates in HT29 colon cancer cells: use of 5-(biotinamido)pentylamine as a transglutaminase-specific probe," *Biochimica et Biophysica Acta*, vol. 1136, no. 1, pp. 12–16, 1992.

[126] Y. Ando, S. Imamura, M. K. Owada, and R. Kannagi, "Calcium-induced intracellular cross-linking of lipocortin I by tissue transglutaminase in A431 cells. Augmentation by membrane phospholipids," *The Journal of Biological Chemistry*, vol. 266, no. 2, pp. 1101–1108, 1991.

[127] N. A. Robinson and R. L. Eckert, "Identification of transglutaminase-reactive residues in S100A11," *The Journal of Biological Chemistry*, vol. 273, no. 5, pp. 2721–2728, 1998.

[128] J. M. Orban, L. B. Wilson, J. A. Kofroth, M. S. El-Kurdi, T. M. Maul, and D. A. Vorp, "Crosslinking of collagen gels by transglutaminase," *Journal of Biomedical Materials Research A*, vol. 68, no. 4, pp. 756–762, 2004.

[129] P. J. T. A. Groenen, H. Bloemendal, and W. W. de Jong, "The carboxy-terminal lysine of αB-crystallin is an amine-donor substrate for tissue transglutaminase," *European Journal of Biochemistry*, vol. 205, no. 2, pp. 671–674, 1992.

[130] P. J. T. A. Groenen, J. J. Grootjans, N. H. Lubsen, H. Bloemendal, and W. W. de Jong, "Lys-17 is the amine-donor substrate site for transglutaminase in βA3-crystallin," *The Journal of Biological Chemistry*, vol. 269, no. 2, pp. 831–833, 1994.

[131] G. A. M. Berbers, R. W. Feenstra, R. van den Bos, W. A. Hoekman, H. Bloemendal, and W. W. de Jong, "Lens transglutaminase selects specific β-crystallin sequences as substrate," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 81, no. 22, pp. 7017–7020, 1984.

[132] S. J. Butler and M. Landon, "Transglutaminase-catalysed incorporation of putrescine into denatured cytochrome. Preparation of a mono-substituted derivative reactive with cytochrome c oxidase," *Biochimica et Biophysica Acta*, vol. 670, no. 2, pp. 214–221, 1981.

[133] S. N. P. Murthy, J. H. Wilson, T. J. Lukas, Y. Veklich, J. W. Weisel, and L. Lorand, "Transglutaminase-catalyzed crosslinking of the Aα and γ constituent chains in fibrinogen," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 1, pp. 44–48, 2000.

[134] J. van den Akker, E. VanBavel, R. van Geel et al., "The redox state of transglutaminase 2 controls arterial remodeling," *PLoS ONE*, vol. 6, no. 8, Article ID e23067, 2011.

[135] S. Orru, M. Ruoppolo, S. Francese, L. Vitagliano, G. Marino, and C. Esposito, "Identification of tissue transglutaminase-reactive lysine residues in glyceraldehyde-3-phosphate dehydrogenase," *Protein Science*, vol. 11, no. 1, pp. 137–146, 2002.

[136] E. Ballestar, C. Abad, and L. Franco, "Core histones are glutaminyl substrates for tissue transglutaminase," *The Journal of Biological Chemistry*, vol. 271, no. 31, pp. 18817–18824, 1996.

[137] A. J. L. Cooper, K. R. Sheu, J. R. Burke et al., "Transglutaminase-catalyzed inactivation of glyceraldehyde 3-phosphate dehydrogenase and α-ketoglutarate dehydrogenase complex by polyglutamine domains of pathological length," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 23, pp. 12604–12609, 1997.

[138] E. Verderio, C. Gaudry, S. Gross, C. Smith, S. Downes, and M. Griffin, "Regulation of cell surface tissue transglutaminase: effects on matrix storage of latent transforming growth factor-β binding protein-1," *Journal of Histochemistry and Cytochemistry*, vol. 47, no. 11, pp. 1417–1432, 1999.

[139] L. K. Rasmussen, L. Ellgaard, P. H. Jensen, and E. S. Sørensen, "Localization of a single transglutaminase-reactive glutamine in the third domain of RAP, the α2-macroglobulin receptor-associated protein," *Journal of Protein Chemistry*, vol. 18, no. 1, pp. 69–73, 1999.

[140] S. N. P. Murthy, J. H. Wilson, T. J. Lukas, J. Kuret, and L. Lorand, "Cross-linking sites of the human tau protein, probed by reactions with human transglutaminase," *Journal of Neurochemistry*, vol. 71, no. 6, pp. 2607–2614, 1998.

[141] L. Eligula, L. Chuang, M. L. Phillips, M. Motoki, K. Seguro, and A. Muhlrad, "Transglutaminase-induced cross-linking between subdomain 2 of g-actin and the 636–642 lysine-rich loop of myosin subfragment 1," *Biophysical Journal*, vol. 74, no. 2, pp. 953–963, 1998.

[142] D. Aeschlimann, M. Paulsson, and K. Mann, "Identification of Gln726 in nidogen as the amine acceptor in transglutaminase-catalyzed cross-linking of laminin-nidogen complexes," *The Journal of Biological Chemistry*, vol. 267, no. 16, pp. 11316–11321, 1992.

[143] M. T. Kaartinen, A. Pirhonen, A. Linnala-Kankkunen, and P. H. Mäenpää, "Transglutaminase-catalyzed cross-linking of osteo-pontin is inhibited by osteocalcin," *The Journal of Biological Chemistry*, vol. 272, no. 36, pp. 22736–22741, 1997.

[144] D. Aeschlimann, O. Kaupp, and M. Paulsson, "Transglutaminase-catalyzed matrix cross-linking in differentiating cartilage: identification of osteonectin as a major glutaminyl substrate," *Journal of Cell Biology*, vol. 129, no. 3, pp. 881–892, 1995.

[145] M. T. Kaartinen, S. El-Maadawy, N. H. Räsänen, and M. D. McKee, "Tissue transglutaminase and its substrates in bone," *Journal of Bone and Mineral Research*, vol. 17, no. 12, pp. 2161–2173, 2002.

[146] L. Gorza, R. Menabò, M. Vitadello, C. M. Bergamini, and F. Di Lisa, "Cardiomyocyte troponin T immunoreactivity is modified by cross-linking resulting from intracellular calcium overload," *Circulation*, vol. 93, no. 10, pp. 1896–1904, 1996.