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The role of transglutaminase-2 and its substrates in human diseases

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1. ABSTRACT

The most characteristic enzymatic function of the class of enzymes known as transglutaminases (TG, EC 2.3.2.13) is the formation of covalent bonds between epsilon-amino groups of primary amines (from lysines or others) and the gamma-carboxamine group of glutamine residues of proteins. In the last years, a growing body of evidence indicate that the most interesting member of the TG family, namely the tissue TG (tTG, also called transglutaminase type 2, TG2), possesses more than one catalytic function. In fact, TG2 is able to catalyze a crosslinking reaction, a deamidation reaction and also shows GTP-binding/hydrolyzing and isopeptidase activities. Therefore, it can act on several classes of substrates, ranging from proteins to peptides, small reactive molecules like mono- and polyamines, and nucleotides. Given the broad spectrum of potentially different activities, elucidating the role of TG2 and its substrates in cellular functions and human diseases is a difficult task. In this study we focus our attention on substrates of TG2 and report a number of interesting considerations about their possible interplay in biological processes and involvement in human diseases, including genetic disorders. A significant improvement in understanding this complex *scenario* may come from a "multi-interfaced" approach, by exploiting different bioinformatic tools. Starting from a database of known TG2 substrates and using bioinformatic cross-search among other databases, we generated relational tables from which an involvement of TG2 in several genetic disorders can be hypothesized. Developing new bioinformatic tools and strategies to investigate the role of TG2 in molecular mechanisms underlying human diseases will add new light to this fascinating field of research.

2. INTRODUCTION

The enzyme transglutaminase (TG) was described for the first time in 1957 (1) and thereafter thoroughly studied and characterized (2-5). The name relates to its main function, i.e. the transamidation of glutamine side chains, which often uses the amine group of a lysine side chain, thus making a covalent crosslink between proteins, or primary amino groups belonging to polyamines, therefore able to act as a bridge. Many synonymous or alternative names have also been used in literature to describe the members of the TG family (according to the BRENDA database http://www.brenda.uni-koeln.de/, the following synonymous terms maybe used: factor XIIIa, fibrin stabilizing factor, fibrinoligase, glutaminylpeptide gamma-glutamyltransferase, glutamyltransferase, glutaminyl-peptide gamma-, Laki-Lorand factor, polyamine transglutaminase, R-glutaminyl-peptide:amine gamma-glutamyl transferase, tissue transglutaminase, transglutaminase, transglutaminase C, TGC, TGase-2, TG2). This large number of synonymous terms reflects the presence of at least 9 related enzymes, all possessing the transamidating activity, but with different localization or substrate specificity. Among the family members, tissue TG (TG2) is probably the most interesting one for different reasons:

- it shows several enzymatic functions;
- it has many different substrates (proteins, nucleotides, amines, drugs), further broadening its spectrum of action;
- although it was originally defined as “tissue” or “cytosolic” TG, it may be also found in extracellular or nuclear environment and may act in different sites by recognizing different substrates;
- some of the chemical reactions catalyzed by this member of TG family have been related to human diseases, opening new pathogenetic perspectives and possibly new therapeutic strategies.
- most recently, TG2 has been involved in biotechnological applications in food chemistry and pharmaceutical fields.

The aim of the present article is to highlight the possible role of this enzyme in human diseases, looking from a specific point of view, i.e. its substrates.

3. METHODS

Bioinformatic tools have been used to search for relationships among TG2, its substrates and genetic disorders. To this aim, four databases have been used:

- TRANSIT (6), which is a specific database of transglutaminase’s substrates, accessible to the web site http://bioinformatica.isa.cnr.it/ASC/. For the present study, the search has been restricted to the TG2 substrates only.
- UniProt, the central database of protein sequence and function created by joining the information contained in Swiss-Prot, TrEMBL, and PIR;
- PubMed, the collection of article abstracts;
- OMIM, the Online Mendelian Inheritance in Man database.

While TRANSIT has been used as a local resource, the other databases where searched by means of the web interface and tools of the SRS Sequence Retrieval System at the EBI web site (http://srs.ebi.ac.uk). By using the cross references among the databases, we created a number of tables containing the relationships among the TRANSIT entries, the UniProt accession numbers of the TG2 substrates, the related entries in OMIM and PubMed references. Some manipulation of the tables has been needed to filter the results by selecting only well characterized substrates of TG2 and verify the absence of redundant entries. The Table 1 of this article represents the final table obtained by merging the information retrieved.

4. TG2 SUBSTRATES

The number of known enzymatic functions of TG2 is growing in the last years, therefore a complete description of the substrates for all these functions may be difficult. Nevertheless, it is possible to cluster the different substrates according to their different chemical properties and different enzymatic reactions involved in. This “clusters of functions” scheme is shown in Figure 1, while Table 1 reports the list of the main substrates so far described, for each chemical reaction catalyzed by TG2. The intersections of different clusters, due to the substrates subjected to different chemical modifications, suggests a very complex TG2 function. This may explain why, until now, its biological functions are still unclear and subject of intense research.

In order to clarify such *scenario*, since 1997 we created an Internet web site (http://crisceb.unina2.it/what/) specifically devoted to improve and spread the knowledge and research on the different members of TG family. It is called W.H.A.T., an acronym standing for Worldwide Happening Around Transglutaminase. More than 18,000 contacts to this web site confirm a large interest in this enzyme, and discussions and exchange of information promoted by WHAT are contributing their part to this field of research.

From structural point of view, the specificity of chemical environment surrounding the reactive glutamyl and lysil side chains is still under investigation (7-12). Recently, novel bioinformatic tools to address these issues have been developed (6).

A list of known substrates for all the TGs has been recently published (13) but newly identified substrates are continuously reported. Here, we summarize in Table 1 the TG2 substrates, including new substrates recently reported in literature, and the specific involvement of these proteins in cellular functions and human pathologies. The following paragraphs describe in more details the possible role of TG2.

Table 1. TG2 substrates and their possible involvement in human diseases

Substrate	Involvement in	OMIM gene entry (accession number)	OMIM genetic disorders entries (with accession number and bibliographic references)	References
Acetylcholine esterase	ND			138
Actin	CR			38-40
Aldolase	GD, MED, OTH	103850	Aldolase A deficiency and myopathy 103850 (139-140)	141
Amines (monoamines, diamines, polyamines): cadaverine, histamine, putrescine, serotonine, spermidine, spermine	CLD OTH			142
beta amyloid peptide	ND	104760	Alzheimer 104300 Cerebral hemorrhage with amyloidosis 609065 (143)	111
Androgen receptor	MED	313700	androgen insensitivity 300068	55
Annexin I (lipocortin I)	OTH, CR	151690		51
alpha(2)-antiplasmin		262850		144
Aspartyl protease	HIV			145
Band 3	MF			43
Band 4.1	MF	130500	Elliptocytosis 130500	43
Calgizzarin - S100C protein - MLN 70 – S100A11	MED, DD	603114		146
Calpactin I light chain (S100A10)		114085		147
CD38	MF	107270		46
Cell adhesion molecule C-CAM	ECM-S			148
Clathrin heavy chain	MF	118955		36
Clara Cell p10 Kda				149
Collagen alpha 1(III)	ECM-S, OTH	120180	Ehlers-Danlos Syndrome type III 130020 and type IV 130050 Acrogeria Gottron Type 201200 (150)	67 151
alpha B-crystallin	CLD, CR, PS	123590	Alexander disease 123590 alpha-B crystallinopathy 608810 (152)	153-154
beta A3 crystallin	CLD, CR, PS			155-156
beta B3 crystallin	CLD, CR, PS	123630		155
beta Bp (betaB2) crystallin	CLD, CR, PS	123620	Sutural cataract with punctate and cerulean opacities 607133 (157)	155
Cystatin 6 (M/E)	CB, DD, OTH	601891	harlequin ichthyosis 242500 (158-159)	160
Cytocrome c	CLD			161
Dual leucine zipper-bearing kinase	ST			48
eIF5A (initiation factor 5A)		600187		162
beta-endorphin	MED, Obesity	176830		163
ERM binding phosphoprotein		604990		36
Fatty acid synthase	MED	600212		36,92
Fibronectin	ECM-S, PS			167
Fibrinogen A alpha	ECM-S, OTH	134820	Amyloidosis, familial visceral 105200 Afibrinogemia 202400 (70)	164-166
Galectin 3	ECM-S, CB	153619	Rheumatoid arthritis 180300 (168)	54,169
Glucagon	MED	138030	Hypoglycemia due to Glucagon deficiency 231530 (170)	171
Glutathione S-transferase				83,172-173
Gluten proteins (alpha/beta-, gamma-gliadin, and low molecular weight glutenin)	CD			100,174-177
Glyceraldeheyde 3 phosphate dehydrogenase	ND	138400		178-179
gp41	HIV			180
gp120	HIV			56
small GTPases	ST, CLD, CB, ND			25
Heat shock proteins	PS, ND	140550 118190 140571		181 36 182
Hepatitis C virus core protein	IF			183
H3 histone	CLD	602820		18
H4 histone	CLD	142750		18
H2A histone	CLD			18
H2B histone	CLD			18
alpha2 HS-glycoprotein (AHSG)				81
Huntingtin	ND	143100	Huntington disease 143100 (184)	185
Importin alpha3	CLD	602970		37
Insulin (A and B chain)	MED	176730	Diabetes mellitus, hyperproinsulinemia 176730	171
Insulin-like growth factor-binding protein-1	MED, ST	146730		50
Insulin-like growth factor binding protein-3 (IGFBP-3)**(a)**	MED, ST	146732		186
Keratin, type II cytoskeletal 1	DD, CB, MF	139350	Bullous ichthyosiform erythroderma 113800 Curth-Macklin type ichthyosis hystrix 146590 Nonepidermolytic palmoplantar keratoderma 600962 Ciclic ichthyosis with epidermolytic hyperkeratosis 607602 Keratosis palmoplantaris striata III 607654 (187-188)	189
Keratin, type II cytoskeletal 2 epidermal	DD, CR, MF	600194	Ichthyosis bullosa 146800 (190-191)	189
Keratin, type II cytoskeletal 5	DD, CR, MF	148040	Epidermolysis bullosa 148040 (192)	189
Keratin, type II cytoskeletal 6	DD, CR, MF	148041	Pachyonychia congenita 167200 (193)	189
Alpha Ketoglutarate dehydrogenase	MED		Alpha ketoglutarate dehydrogenase deficiency 203740 (194)	178
alpha-Lactalbumin		149750		195-197
beta Lactoglobulin				198-200
Latent TGF-beta binding protein-1 (LTBP-1)	CB, OTH			109, 201
Lipoprotein a				71

Loricrin	DD, MF	152445	Vohwinkel syndrome 604117 (202)	203
alpha2 Macroglobulin	OTH			204
alpha-2-Macroglobulin receptor-associated protein	OTH	104225		205
Mellittin				206
Microtubule-associated protein tau - isoform Tau-F (Tau-4)	CR, ND		Frontotemporal dementia 600274, Pick disease 172700 pallido-ponto-nigral degeneration 168610 Progressive supranuclear palsy (PSP) 601104, 260540 Hereditary dysphasic disinhibition dementia 607485	113
Midkine	OTH, MED			53-54,207
Myelin Basic Protein	ND	159430	Multiple sclerosis 126200	114
Myosin				208
Nidogen (entactin)	ECM-S			65
Nucleotide(s) binding/hydrolyzing	ST			45,209-211
Osteocalcin	OTH			212
Osteonectin	OTH, ECM-S			80
Osteopontin (extracellular matrix cell adhesion protein)	OTH, ECM-S			79,81,213
Periplakin	DD			214
Phosphoglycerate dehydrogenase	MED	606879	Phosphoglycerate dehydrogenase deficiency 601815	36,92
Phosphoglycerate kinase				215
Phospholipase A2	MED, ST, OTH			104
Phosphorylase kinase	MED			216
Plasminogen-activator inhibitor type-2		173390		217-218
Polyglutamine	ND	143100		118
Proapoptotic kinase DLK	CLD			20
Procarboxypeptidase U (EC 3.4.17.20) plasma procarboxypeptidase B		603101		219
Retinoblastoma protein	CLD			19
Rho A	ST			22
Ribonuclease A				134
S100 calcium-binding protein A7 - Psoriasin (S100A7 or PSOR1)	DD	600353		147
Seminal vesicle secretory protein IV	Fertility OTH			220
Sialoprotein (BSP)				81
Soybean proteins				221
Spectrin alpha	MF	182810		27,36
Substance P	ND, OTH	162320		222
Synapsin I	CR, ND			42
alpha-Synuclein	ND	163890	Alzheimer disease 104300 (143,223)	112, 224
Thymosin beta 4	CS, OTH			39
Thyroglobulin	MED	188450		225
Tissue Transglutaminase	CD	190196		100
Troponin T	CR			87
Tubulin	CR			41
Uteroglobin	OTH MED			226
Valosin		601023		36
Vasoactive intestinal peptide (VIP)	OTH, MED	192320		122
Vimentin	CR			88
Vitronectin	OTH, ECM-S			69

Note: Published and well characterized substrates of TG2 enzymatic functions have been listed showing their known, or hypothesized, involvement in the following cellular regulation processes and human diseases: Protein Stabilization (PS), membrane traffic and membrane structure/function (MF), signal transduction (ST), extracellular matrix-cell interaction and stabilization (ECM-S), cytoskeleton regulation (CR), Celiac disease (CD), other autoimmune, inflammatory and related diseases (OTH), neurological diseases (ND), metabolic and endocrinology diseases (MED), Cancer biology (CB), genetic disease (GD), dermatologic diseases (DD), HIV and infectious disease (HIV– IF). [a]**:** substrate of the kinase activity of TG2.

4.1. Involvement of TG2 substrates in cell functions

4.1.1. Cell life and death

Although $TG^{-/-}$ knockout mice did not show significant perturbation in apoptosis (14,15), many reports in the last 15 years showed that TG2 may act as pro-apoptotic protein (16,17). In fact, a large number of substrates have been identified whose covalent modification may be relevant to cell death induction, like cytoskeletal proteins (actin, beta-tubulin), nuclear proteins (e.g. histones (18)), and extracellular matrix proteins (see below). Further, covalent modifications involving polyamines, growth factors and receptors, catalyzed by TG2, have been shown to play a role in programmed cell death cascade. Key modulators of cell survival and death, like retinoblastoma protein (19), the pro-apoptotic enzyme (DAP-like kinase (DLK) (20), may also be crosslinked by TG2 and their functions have been shown to be modulated by this reaction. Further, it was reported that TG2 ablation reduces neuronal death (21).

Another reaction catalyzed by TG2 and possibly related to cell fate is linked to signal transduction and G-proteins. In fact, TG2 itself is a non-canonical G-protein (Gh) which is able to bind and hydrolyze GTP (see below). Further, TG2 can also *in vivo* modify RhoA, a member of Ras superfamily (22) and it was shown that retinoic acid induced transamidation of RhoA is able to promote cytoskeleton rearrangement and activation of MAP kinase pathway (23). RhoA transamidation is also involved in platelet aggregation, exocytosis and cytoskeleton rearrangement (24,25).

4.1.2. Membrane traffic and membrane structure/function

TG2 is relevant to plasma membrane structure and function from several points of view. In erythrocytes, human protein 4.2, a protein closely related to TG2 (3), is a major component of the erythrocyte membrane (26). A reduced covalent modification of spectrin and band 3

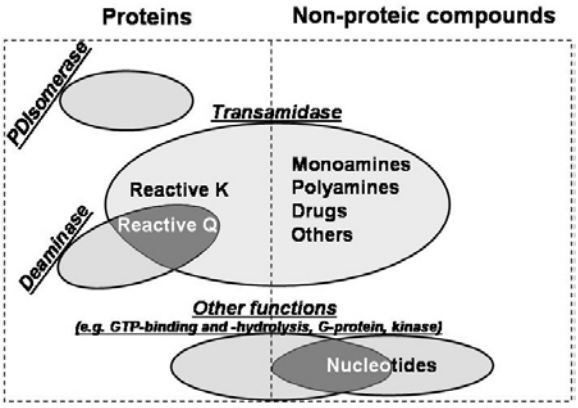

Figure 1. Clustering of enzymatic functions of TG2 and its various substrates.A schematic representation of functions (typed in *italics* and underlined) and substrates of TG2. Reactive K and Reactive Q indicate the modified lysines and glutamines, respectively, in protein substrates.

catalyzed by TG was shown to occur in sickle erythrocytes (2,27). Also, a role of TG2 in regulating structural flexibility of red blood cells was reported (28). Association of protein-4.2 with band 3 and possible relationships with hemolytic anemia have also been recently shown (29).

TG2 has been involved in receptor-mediated endocytosis and phagocytosis (16,30-33). A possible involvement of TG2 in membrane trafficking processes was also previously hypothesized by studies on neurotransmitter release (34-35). Proteins implicated in transport processes like valosin and clathrin have been shown to be glutamine-donor substrates, whereas importin has been shown to be a lysine-donor (36); other studies showed that TG2 can interact with the nuclear transport protein importin-alpha3 (37). Other protein substrates, involved in membrane functions and shown to be TG2 substrates are actin (38-40), tubulin (41), synapsin (42), band 4.1, spectrin and band 3 (43).

4.1.3. Signal transduction

TG2 may significantly interfere with cell fate by affecting signal transduction across the plasma membrane. TG2 is a GTP-binding and hydrolyzing protein Gh (3-4, 44-45). It is involved in the activation of members of the Rho-GTPase family (22,46-48). Transamidation of RhoA induced by retinoic acid leads to association of activated RhoA with Rho-associated coiled-coil-containing protein kinase 2 (ROCK-2), thereby promoting a formation of stress fibers and focal adhesions. Cytoskeletal proteins ezrin / radixin / moesin, intracellular signaling proteins and elongation factors critical for assembly of junctional protein complexes and actin-cytoskeleton organization in intestinal epithelia serve as TG2 substrates and are strongly implicated in the development of celiac disease (36,49). Therefore, TG2 may act as signal transduction protein by altering the signaling function of certain growth / differentiation factors such as CD38 transmembrane enzyme (46), dual leucine zipper-bearing kinase (48), insulin-like growth factor-binding protein 1 (50), lipocortin I (51) and the extracellular midkine (52-54). Many of these proteins are also TG2 substrates *in vivo*. On the other hand, TG2-catalyzed crosslinking of transmembrane proteins or receptors (e.g. androgen receptor (55), gp41 (56)) was also described. This supports a hypothesis that this enzyme may be involved in modulation of signaling across plasma membrane serving as both G-protein and TG catalyzing the formation of stable covalent cross-links among membrane components and/or intracellular effectors.

4.1.4. ECM-cell interaction and stabilization

TG2 lacks a leader sequence, nevertheless it is released from cells into the extracellular space where it has been implicated in extracellular matrix (ECM) stabilization and in cell–ECM interaction by cross-linking matrix proteins (57). TG2 released from cells is tightly bound to fibronectin and collagens, forming ternary complexes that function as a cementing substance in the ECM. This non-covalent interaction was thoroughly investigated and binding sites within TG2 have been characterized (58-63). Fibronectin is considered a major TG2 substrate both *in vitro* and *in vivo* (64). Further, TG2 is able to stabilize heteromeric complexes in the ECM of specific tissues (4,5), like laminin–nidogen (65), fibronectin–collagen (66-68) and osteonectin–vitronectin (69). Other TG2 protein substrates involved in ECM assembly, remodelling and stabilization, are fibrinogen / fibrin (70), lipoprotein(a) (71), laminin and nidogen (65) and galectin-3 (72). Further, other important proteins for ECM structure and functions, like von Willebrand factor (73-74) and vitronectin (75) were shown as substrates for another member of the transglutaminase family (factor XIII). These studies, mostly carried out *in vitro* but some also confirmed *in vivo*, clearly demonstrate a role for TG2 in cell-ECM interactions and ECM stabilization. This role is confirmed by the observation that ECM perturbation related to TG2 function is involved in various liver, renal, dermal diseases, pulmonary fibrosis and atherosclerosis (76), as well as in metastatic cell spreading and invasion, another pathological process dependent on cell-ECM interactions (72,77-78). Furthermore, a role in tissue mineralization by catalyzing the formation of the cross-linked clusters of the Ca^{2+}-binding proteins osteonectin and osteopontin at the cell surface has been reported (79-81).

4.1.5. Regulation of cytoskeleton

Many TG2 substrates are involved in cytoskeleton organization and functions. In fact, in the cytoskeletal compartment, TG2 co-localizes with stress fibers and, through autocatalytic reaction, cross-links itself to myosin. Upon calcium activation, TG2 may control organization of the cytoskeleton by cross-linking various cytoskeletal proteins, i.e. microtubule protein tau (40,82-83), tubulin (84), actin (39), myosin (36), spectrin (36), thymosin (39,85), troponin T (86-87), synapsin I (42) and vimentin (88), histones and nuclear proteins (18), as well as retinoblastoma gene product, a key signal for apoptosis initiation (19). Polymerization of cytoskeletal components occurs during the final steps of apoptosis, stabilizes the structure of the dying cells preventing the leakage of cellular components involved in inflammatory/autoimmune responses (4).

4.2. From the cell to the whole organism: involvement of TG2 substrates in human diseases

Deregulation of TG2 expression and/or function has been proposed to be involved in a number of human diseases, like celiac disease, cancer, diabetes mellitus, some neurological pathologies such as Alzheimer's and Parkinson disease, and others. However, the role of TG2 is at this time well established only for celiac disease, while more investigation is needed about the role of TG2 in other diseases.

4.2.1. Celiac disease

Celiac disease (CD), or celiac sprue, or gluten-sensitive enteropathy, is a multifactorial disorder influenced by both environmental and genetic factors (49,89-92). The inflammatory injury of the small intestine mucosa, occurring in celiac patients after the ingestion of wheat gluten or related rye and barley proteins, induces a flattening of intestinal epithelium, leading to an inefficient uptake of nutrients. Symptoms include diarrhoea, malabsorption, and failure to thrive. At present the only effective treatment for the disease is the removal of gluten from the diet, and the reintroduction of gluten in the patient's diet invariably leads to the reappearance of the symptoms. The molecular basis of CD is still unclear, however the molecular mechanism is considered to start with the binding of gluten peptides to HLA molecules and then the specific recognition by T cells (90). HLA-DQ2 (DQA1*0501/B1*0201) is found in the great majority of CD patients, while DQ8 (DQA1*0301/B1*0302) is found in most of the remaining patients (89-90). Different gluten-derived peptides are recognized by T cell clones isolated from biopsies of CD patients (93-95). The intestinal T cell response to alpha-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by TG2 (95). The binding of gluten peptides to DQ2 and DQ8 molecules has been experimentally observed. It may be increased by the presence of negatively charged amino acids at known positions in the peptide (89). Gluten proteins are very rich in glutamine and proline and contain very few negatively charged amino acids, but glutamines may be deamidated to the negatively charged glutamate residues by TG2, with the consequence of improving both the binding to DQ2 and the response of T-cell clones (93,95-97). It is also known that celiac patients treated with a gluten-containing diet produce immunoglobulin (Ig)A and IgG autoantibodies specific for TG2 (98).

Therefore, the function of TG2 in celiac disease appears to be related to deamidation of glutamine side chains, reaction preferred to transamidation when the pH decreases (99). In addition, it has been reported that gluten peptides incubated with TG2 create covalent complexes via a thioester bond to the active site cysteine of TG2 as well as via isopeptide bonds to particular lysine residues of the enzyme (100). Therefore, gluten proteins and their derived peptides represent substrates of different TG2-catalyzed reactions.

4.2.2 . Other autoimmune, inflammatory and related diseases

The presence of autoantibodies against TG2 as well as other substrates in autoimmune diseases like celiac disease suggests that TG2 may cross-link potential autoantigens to itself and to other protein substrates: the TG2–protein complexes formed *in vivo* may therefore function as hapten–carrier complexes (101). This reaction might then trigger an immunological response typical for autoimmune diseases (76,101). In fact, an immune reaction was observed against the known TG2 substrates actin, lipocortin I, myosin, tubulin, and histone H2B in patients with systemic lupus erythematosus, against collagen and myelin basic protein in bullous pemphigoid and multiple sclerosis, respectively (76), and against TG2 itself in Sjogren's syndrome (102-103). A role of TG2 in inflammation diseases is also considered to be related to its regulatory action on granule secretion and macrophage function (see above), or acting on the function of key inflammation mediators like phospholipase A2 (104). An involvement of TG2 in inflammatory diseases and related processes like angiogenesis and wound healing has been reported (105-106). It is also considered important in pathogenesis of chronic inflammatory diseases such as rheumatoid arthritis and osteoarthritis through regulation of availability of the latent transforming growth factor binding protein-1 in the matrix, given that its TG2-catalyzed crosslinking to the matrix induces the release of the active transforming growth factor beta (107-109).

4.2.3. Neurological diseases

A large body of evidence is available regarding the role of TG2 in neurodegenerative disorders like Alzheimer, Parkinson and Huntington diseases (76,110). Several protein substrates have been shown to be *in vitro* and/or *in vivo* TG2 substrates in the neuron cellular compartments, e.g. amyloid beta-A4 peptide (111), alpha-synuclein (111-112), the microtubule-associated tau protein (113), synapsin I (42), as well as myelin basic protein (114). Aggregates containing these proteins have been found *in vitro* and/or in extracellular compartments in the CNS of patients suffering from degenerative neurological diseases. Also**,** a possible involvement of TG2 in neurotransmitter release and related pathological processes like tetanus neurotoxin intoxication, has been shown (35,115-116). Therefore, TG2-mediated cross-linking is believed to be implicated in the pathogenesis of Alzheimer disease, Parkinson disease and in progressive suprabulbar palsy (76,110) and hypothetically in the diseases related to neurotransmitter release (34,117). Further, TG2 crosslinking activity is also considered important in the Huntington disease, a neurological disease characterized by the presence of expansion of CAG repeats (polyglutamine expansion disease) (21,118-120). The neurodegenerative process leading to cell apoptosis may also be due to covalent modification of TG2 substrates involved in energy metabolism like GAPDH and alpha-ketoglutarate dehydrogenase (47).

4.2.4. Metabolic and endocrinologic diseases

With regard to the involvement of TG2 in metabolic homeostasis, it is noteworthy that besides the GAPDH and alpha-ketoglutarate dehydrogenase mentioned above, phosphoglycerate dehydrogenase and fatty acid synthase are also TG2 substrates *in vitro* (36,92). In addition, TG2-dependent covalent modification of several

hormones, receptors or hormone-binding proteins has been reported (50,55,121-122), suggesting that TG2-catalyzed crosslinking may be relevant in controlling complex metabolic responses to hormones action. A possible involvement of TG2 in diabetes mellitus was reported (4,123-125) and it was hypothesized that TG2 has a role in regulation of insulin secretion (126-131).

4.2.5. Cancer biology

An important role of TG2 in cancer biology has been recently reviewed (78). Indeed, TG2 is involved in modulation of cell programmed death and cell fate via many crucial cellular functions, including cell motility, cytoskeleton assembly and function, membrane traffic, signal transduction (see previous sections). Then**,** a strong evidence was also presented about a direct connection of TG2 with drug resistance (132-133), mechanisms of metastatic dissemination (77) and modulation of key signaling cascades in several types of human cancers. Many molecular targets of these TG2-dependent effects are functioning not only as substrates of covalent crosslinking but possibly also as non-covalent interactors, e.g. integrins, laminin, galectin-3, TGF-betą and ECM proteins (72,78,92). Interestingly, TG2 expression may be induced by a number of chemical/physical agents like cyclic AMP, retinoic acid, dexamethasone, tumor necrosis factor, growth factors (e.g. EGF), cytokines and UV radiation. Further, TG2 involvement in carcinogenesis also was hypothesized when stress conditions (e.g, chemotherapy or UV radiations) induce an increase of intracellular calcium ions (78). Several molecules, involved in cancer development, spreading and evolution, such as integrins, galectin-3, ECM proteins, cytoskeletal components, nuclear proteins, such as retinoblastoma protein and E2F, have all been shown to be modified by TG2.

4.2.6. Genetic disorders

The involvement of TG2 in human diseases may be actually greater than expected, if pathologies related to genetic disorders are also considered. Table 1 lists the characterized substrates of TG2, most of which are proteins related to human genetic diseases. It is possible to hypothesize that post-translational modifications induced by TG2 may affect the bio-availability or the function(s) of the target proteins, leading to a pathological condition. To support this hypothesis, a specific search among different databases, i.e. TRANSIT, OMIM (Online Mendelian Inheritance in Man), UniProt and MedLine/PubMed, has been carried out by exploiting bioinformatic tools (see Methods section for details). This search has revealed 87 OMIM entries related to TG2 and its substrates, with 991 PubMed entries referring to these OMIM entries. Table 1 reports the OMIM disorders related to the protein substrates of TG2 and the main references. As shown in the Table, at least 50% of protein substrates are found in an OMIM gene entry and about 20% are related to at least one specific genetic disorder as the consequence of known mutations or defects in expression. A continuous updating of such databases suggests that the number of found entries should increase over the time. Two main conclusions can be made. (I) The relationships between TG2 and genetic disorders reported in the Table have been found by searches based on the cross-references among databases, so it is possible that the contents of PubMed articles do not reflect direct relationships between TG2 and that specific disease. Of course, a direct validation of the found relationships needs to be performed by biochemical and/or clinical studies. And, (II) despite the fact that TG2 is the most studied member of the TG family, many proteins have been tested as substrates of TGs in general, so it may be possible that some diseases might be related to different members of the family.

4.3. Other substrates for other functions

TG2 is able to catalyze more than one enzymatic reaction, e.g. transamidase, deaminase, GTPase, protein kinase, protein disulphide isomerase (PDI) (2, 13, 134, 186). Therefore, TG2 may not only crosslink specific protein substrates, but also catalytically modify mono- and poly-amines, nucleotides and proteins as substrates for deamidation, PDI-reaction and phosphorylation (see Figure 1). On the other hand, besides the transamidation reaction involving glutamine and lysine residues of various proteins, TG2 is also likely to catalyze the covalent incorporation of polyamines into specific acyl-donor substrates. This reaction may structurally and functionally modify other proteins, or enzymes, triggering a cascade of subsequent effects. For instance, TG2-catalyzed polyamination of phospholipase A2 increases its enzymatic activity *in vitro* (104), polyamination of microtubule-associated protein tau significantly inhibits its calpain-mediated proteolysis (82), substance P covalently modified by spermine and spermidine incorporation protects the peptide against proteolysis (135). Further, a polyamine-activated TG2 was also shown to be directly responsible for spermidine- and spermine-induced apoptosis in human vascular and melanoma cells (136). On the other hand, since the majority of GTP-binding activity detected in rabbit liver nuclear preparations was due to TG2 (137), its activation as GTP-binding and GTP-hydrolyzing enzyme may play a crucial role in controlling intracellular concentrations of nucleotides and, consequently, the fine-tuning of many signal pathway(s). Indeed, the regulation of key molecules such as nucleotides and polyamines within the cell may represent an additional explanation of the role that TG2 plays in controlling cell functions, such as cell death and differentiation, and human diseases, such as cancer. Therefore, the role of TG2 in the regulation of intracellular concentrations of mono/polyamines, and GTP and other nucleotides, represents a field of investigation which, in our opinion, deserves a further study.

Finally, as shown in this and other studies, TG2 can modify the functions of several other enzymes (see Table 1), as a consequence acting on a variety of other substrates. Therefore, it should be highlighted that the involvement of TG2 in normal cellular functions and in pathogenesis of human diseases may be due to both direct and indirect effects.

5. CONCLUSIONS AND PERSPECTIVES

In conclusion, we believe that several lines of evidence show a key role of TG2 in controlling different cellular functions. This is due to many different substrates

that TG2 may recognize and modify. Both experimental studies and bioinformatic analyses allow to hypothesize that besides the known involvement in pathogenesis of celiac disease, TG2 may be profoundly involved in several human diseases, including some genetic disorders. The lack of potent and specific inhibitors of this particular member of the TG family represents a technical limitation which, at this time, makes it difficult to more accurately evaluate its role in human diseases. Another limitation is related to several related members of TG family with overlapping biological functions, which makes the results obtained with murine knock-out models rather inconclusive.

Developing new inhibitors and other *in vivo* models will lead to more precise evaluation of the role of TG2 in pathology; more flexible bioinformatic tools and better annotated databases will make possible more detailed searches and logical links to experimental data, giving a more complete view of the role of TG2 in human disorders.

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