

Transglutaminase 2 and Anti Transglutaminase 2 Autoantibodies in Celiac Disease and Beyond: TG2 Double-Edged Sword: Gut and Extraintestinal Involvement

Aaron Lerner^{1,2*}, Sandra Neidhöfer² and Torsten Matthias²

¹Rappaport School of Medicine, Technion-Israel Institute of Technology, Haifa, Israel

²Aesku Kipp Institute, Mikroforum ring 2, Wendelsheim 55234, Germany

*Corresponding author: Aaron Lerner, Aesku. Kipp Institute, Mikroforum ring 2, Wendelsheim 55234, Germany, Tel: 49-6734-9622-1010; Fax: 49-6734-9622-2222; E-mail: aaronlerner1948@gmail.com

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Abstract

Transglutaminase2 is a pleiotropic enzyme expressed ubiquitously and abundantly. It has been implicated in a variety of physiological processes, such as growth, differentiation, migration, signaling, cytoprotection, cell death and survival, wound healing, angiogenesis, inflammation, apoptosis and autophagy. It operates intracellularly in multiple organelles, extracellularly and on cell surface. Apart from catalyzing post-translational modifications of proteins, by deamidation and cross-linking, it exercises G-protein, isomerase and kinase activities and non-enzymatic biological functions. Aberrant activation or deregulation of its functions is involved in numerous human disease. The most known one is celiac disease, but the present review will expand on extraintestinal entities. It plays a role in inflammatory, degenerative-age related, neurodegenerative, malignant, metabolic and hormonal, autoimmune and genetic conditions. Increased knowledge of its structure, functions and regulation in homeostatic phase, open the opportunity to design new therapeutic strategies to inhibit its malfunction in pathological situations.

Keywords: Tissue transglutaminase; TG2; Function; Pathology; Tissue; Celiac disease; Gut; Extraintestinal manifestation

Abbreviations

TG2: Transglutaminase2; ECM: Extracellular Matrix; SLE: Systemic Lupus Erythematosus; CFTR: Cystic Fibrosis Transmembrane Conductance Regulator; MODY: Maturity Onset Diabetes of the Young

Characteristics of Physiologic Transglutaminase2 (TG2)

Transglutaminase (Enzyme Commission [EC] no. 2.3.2.13, OMIN[†]190196), i.e., protein-glutamine γ -glutamyltransferase, belongs to the class of transferases. It catalyzes the formation of an isopeptide bond between the group of γ -carboxamides of glutamine residues (donor) and the first-order ϵ -amine groups of different compounds, for instance, proteins (acceptors of an acyl residue). The human TG2 gene is localized to chromosome 20q11-12, the protein is made up of 687 amino acids and it is called also tissue TG. It is the most abundant and most studied of the nine members of the TG enzyme family [1].

Three reactions are catalyzed by transglutaminase: an acyl-transfer reaction, a crosslinking reaction between Gln and Lys residues of proteins or peptides (transamidation), and deamidation. If lysine is the acceptor of an acyl group, then a protein molecule is enriched with this amino acid. The transfer of an acyl group onto a lysine residue bound in the polypeptide chain induces the process of crosslinking. In addition, transglutaminase catalyzes the reaction of deamination if there is an absence of free amine groups. The reactions catalyzed by this enzyme result in significant post translational modification and

changes in the physical and chemical properties of proteins, such as modifications in the viscosity, thermal stability, elasticity, and resilience [2]. Beside the primary TG enzymes' activity of catalyzing the calcium-dependent post translational modifications, it can also bind and hydrolyze GTP, exhibit protein disulphide isomerase and kinase activities, independently of calcium and mediates trans-membrane signal transduction and interactions between cell surface proteins and the extracellular matrix. It can interact with a number of cell surface proteins, in a non-enzymatic way, taking part in cell adhesion pathways and extracellular matrix stabilization [2]. 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, thus preventing inflammation to develop [1,3]. This highly complex multifunctional enzyme and due to its pivotal importance in cell biology, the catalytic activity of TG2 is tightly controlled and nature has evolved an intricate scheme of allosteric ligands and modifications to induce or inhibit the enzyme [4]. Extracellular TG2 has a role in cell adhesion and extracellular matrix organization, whereas intracellular TG2 is involved in signaling events leading to regulation of cell survival, particularly in response to cell wounding, hypoxia, and oxidative stress. It is a major regulator of endothelial cell proliferation and apoptosis [5].

TG2 Tissue Localization

Transglutaminases are widespread in nature. They are found in mammalian tissues, in many invertebrates, in plants, and in microbial cells. Microbial TG is used heavily in the industrial food processing, acting as a protein glue, and was most recently suggested as an inducer of celiac disease [6,7]. TG2 is ubiquitously expressed in endothelial cells, fibroblasts, osteoblasts, smooth muscle cells, monocytes, neutrophils, neurons, chondrocyte, oligodendrocyte, dendritic cells,

epithelium, stem cells and macrophages. It spans the intracellular compartments and organelles like: nucleus, mitochondria, endoplasmic reticulum, cytoskeleton, and cytoplasm and on the cell surface and in the extracellular compartment [8]. It is involved in numerous human biological events. The vast array of biochemical activities of TG2 accounts for its involvement in a variety of cellular processes, including adhesion, migration, growth, survival, apoptosis, differentiation, exocytosis, autophagy, cytoprotection and extracellular matrix organization [9].

Genetic Aberrations in TG2 Gene

Unlike in the case of most other TGs there are no common single nucleotide polymorphisms in the exon of human TG2. Four mutations and transcriptional variants were described for TG2. One lacks the C-terminal 139 and is unresponsive to GTP and is named TGH. It is up regulated in Alzheimer's diseases and apoptotic conditions. The second variant (TGH2) lacks the C-terminal 338 amino acid and has an altered sequence in its terminal residues 287-349, likely jeopardizing its catalytic activity. The two others (tTgv1, tTGv2) have divergent 54 or 25 amino acid C-terminal sequences, compared to TG2 [4].

Several missense mutations were described in the TG2 gene, implicated in glucose intolerance such as early onset type 2 diabetes or MODY. Some of those functional mutations map to the vicinity of the putative Ca²⁺ binding site, resulting in modest reduction of the calcium dependent transamidation activity of the enzyme. Based on functional studies of the rare protein variants, found so far only as heterozygous forms, the amino acid changes lead to defective proteins. However, it will be very difficult to detect pathological entities, considering their very low frequency of occurrence. Despite a strong evolutionary pressure on the human TG2 gene, the human mutations with subtle, if any, phenotype, may explain the normal development and reproductivity of the TG2-null mice [4,10]. In fact, although there are more than 300 TG2 SNPs in the normal populations, there is no report of their association with any disease. It is conceivable that a deficient activity of the enzyme develop in a non-genetic way, contributing to disease progression [10].

TG2 Involvement Non-Celiac Human Diseases

TG2 impact on the above mentioned fundamental processes implicate the enzyme in numerous pathological conditions. TG2 is involved in many human diseases affecting wound healing, inflammation, autoimmunity, tissue fibrosis, tumor growth, vascular remodeling, cancer and metastasis and neurodegenerational conditions [9]. In most of those diseases the role of TG2 is mostly related to the dysregulation of its functions, mainly regarding cellular matrix interaction and stabilization, rather than its apoptotic involvement [1].

Inflammatory diseases

The pivotal role of TG2 in regulation of granule secretion, macrophage functions and regulation of inflammatory mediators, drive the inflammation [1]. TG2 is involved in angiogenesis and wound healing [11]. In chronic inflammatory diseases like: rheumatoid arthritis, osteoarthritis by activating TGF- β . It is considered as a biomarker of osteoarthritis and regulator of cartilage destruction and osteophyte and invadopodia formation [12-14]. Most recently TG2 was found to be essential for adherence of porphyromonas gingivalis (an environmental inducer of rheumatoid arthritis) to host cells [15]. In

chronic kidney disease through the enhancement of matrix vesicle-extracellular matrix interaction. In cystic fibrosis, the generation of an oxidative stress induced by CFTR-defective function leads to protein inhibitor of activated STAT (PIAS) γ -mediated TG2 SUMOylation and inhibits TG2 ubiquitination and proteasome degradation, leading to sustained TG2 activation. TG2, is an enhancer of the inflammation in alcoholic steatohepatitis. Recently, a novel role of TG2 was observed in allergic inflammation and suggested as a target for the development of allergy therapeutics.

Chronic degenerative diseases

TG2 plays a crucial role in the formation of insoluble protein aggregates and has been implicated in the pathogenesis of many diseases termed "conformational disease". It contribute to the formation of crystalline polymers in senescent cataracts and other age-related diseases. Aging of the extracellular matrix (ECM), the protein matrix that surrounds and penetrates the tissues and binds the body together, contributes significantly to functional aging of tissues. ECM proteins become increasingly cross-linked with age, and this cross-linking is probably important in the decline of the ECM's function. Extracellular TG2 is therefore therapeutic target both for specific and more generalized diseases of aging. Protein aggregation is in the basis of multiple neurodegenerative conditions' progression [1].

Neurodegenerative conditions

Many TG2 substrates are found in the neuronal cellular compartments, and the local enzyme activity induces protein aggregation, recently found to be mediated by endoplasmic reticulum stress [16]. TG2 is considered a significant disease-modifying factor in neurodegenerative diseases because TG2 might enzymatically stabilize aberrant aggregates of proteins implicated in these diseases. However, the causal role of TG2 in Ca²⁺ signaling in brain pathogenesis has been unclear. TG2 contribute to aggregation of huntingtin protein in Huntington's disease, accumulation of insoluble neurofibrillary tangles and β -amyloid plaques in Alzheimer's disease, or α -synuclein in Parkinson's disease progression. The enzyme is additionally involved in neurotransmitter release conditions like the botulinum and tetanus neurotoxins mode of action.

Malignant diseases

TG2 has been shown to play a major role in cancer genesis and metastatic progression and spreading. When aberrantly regulated, the enzyme help the tumor cells to evade apoptosis, resulting in cancer drug resistance. High expression of TG2 promotes stem cell phenotype suppressing terminal differentiation of cancerous cells. TG2 is implicated in numerous cancerous conditions like: ovarian, pancreatic, breast, melanoma, lung, and glioblastoma. In some condition TG2 expression serve as a predictor of cancer behavior and prognosis or reactivity to specific drug therapies [1].

Metabolic disease

The covalent modifications induced by TG2 acting on key enzymes involved in energy production and metabolism, account for its role in multiple metabolic diseases. Glyceraldehyde -3-phosphate dehydrogenase, alpha-ketoglutarate, phosphoglycerate dehydrogenase and fatty acid synthase deficiencies are some of them. In addition TG2 mediate covalent modifications of hormone receptors or binding proteins thus impacting hormonal complex metabolic responses.

Involvement in insulin secretion and post transcriptional modifications of protein catalyzed by TG2 was suggested recently, thus involving the enzyme in type 1 diabetes mellitus [1,17]. The involvement of TG2 in type 2 diabetes and MODY was describe above. Most recently, the role of TG2 in diabetic cardiomyopathy was unraveled and rutin was suggested as a therapeutic drug to inhibit the expression of the enzyme, to regulate myocardial damage [18].

Autoimmune diseases

An addition to celiac disease, which is out of the scope of the present review and type 1 diabetes discussed above, TG2 is involved in several other autoimmune diseases. In a wild-type mice, treatment with low-molecular weight TG-inhibitor ameliorate multiple sclerosis disease severity and in human, TG2 is present in astrocytes in the disease lesions [17]. TG2 is also involved in SLE like syndromes, by its effects on apoptosis [19]. In fact, an immune reaction was observed against the known TG2 substrates actin, lipocortin, myosin, tubulin and histone H2B in patients with SLE, against collagen and myelin basic protein in bullous pemphigoid and multiple sclerosis, respectively, and against TG2 itself in Sjogren's syndrome. It is quiet unusual that both gain and loss of function disturbances of TG2 are leading to autoimmunity. In celiac disease, TG2 is the initiator and the target of the disease and is associated with increased apoptosis. On the other spectrum, lack of TG2 in mice results in classical autoimmune

disease involving decreased clearance of apoptotic cells. However, autoimmune diseases as a result of deficient TG2 activity has not been observed in human [19].

Genetic diseases

The balance between import from the cytoplasm to the nucleus, and export from the nucleus to the cytoplasm, determines the level of TG2 in the nucleus. Selective regulation of the expression, activity or localization of nuclear TG2 play a central role in genetic aberrations and opens a new era for this long-studied enzyme. An extended search in different databases revealed 87 OMIM entities, related to the protein substrates of TG2 [20]. At least 50% of them 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. Just to enumerate some of them: elliptocytosis, Ehlers-Danlos syndrome type III, harlequin ichthyosis, ichthyosis bullosa, glucagon deficiency, pachyonychia congenital, α ketoglutarate dehydrogenase deficiency, phosphoglycerate dehydrogenase deficiency, alkaptinuria, Huntington's, recessive dystrophic epidermolysis bullosa, and cystic fibrosis [20]. Table 1 summarizes the diseases' categories and specific human conditions involving TG2 dysfunctions. Figure 1 describes schematically the TG2 domains and its involvement in human diseases.

| Disease type | Specific condition | References |
|-------------------|---|------------------------|
| Inflammatory | Rheumatoid arthritis, osteoarthritis, chronic kidney disease, cystic fibrosis, alcoholic steatohepatitis, allergic inflammation | [11-15,2] |
| Degenerative | Senescent cataracts, functional aging of tissues | [1,20] |
| Neurodegenerative | Huntington's disease, Alzheimer's, Parkinson's disease | [16,20] |
| Malignant | ovarian, pancreatic, breast, melanoma, lung cancers and glioblastoma | [1,20,21] |
| Metabolic | Type 1 and 2 diabetes mellitus, MODY, diabetic cardiomyopathy, glyceraldehyde -3-phosphate dehydrogenase, alpha-ketoglutarate, phosphoglycerate dehydrogenase, fatty acid synthase deficiencies | [1,17,18,20] |
| Autoimmune | Type 1 diabetes, Celiac disease, Dermatitis herpetiformis, multiple sclerosis, SLE, bullous pemphigoid, Sjogren's syndrome, rheumatoid arthritis | [2,6,7,11,13-15,17,19] |
| Genetic | Elliptocytosis, Ehlers-Danlos syndrome type III, harlequin ichthyosis, ichthyosis bullosa, glucagon deficiency, pachyonychia congenital, α ketoglutarate dehydrogenase deficiency, phosphoglycerate dehydrogenase deficiency, alkaptinuria, Huntington's, recessive dystrophic epidermolysis bullosa, cystic fibrosis | [1,20] |

Table 1: Transglutaminase2 involvement in human diseases.

TG2 as A Novel Therapeutic Modality in Non-Celiac Human Diseases

TG2 enzyme has two faces: pivotal in cell functions and survival or inducer of neopeptides resulting in inflammation and autoimmunity. Due to its pivotal role in pathogenesis of multiple diseases, it is not astonishing that it is a logical target for future therapeutical strategies [9]. In this regard, several approaches were perused: 1. Inhibition of transamidation/cross-linking activity, in neurodegeneration and fibrosis, by irreversible inhibition binding of the active site cysteine; 2. The improved motor function and increased survival of cystamine-treated mice with Huntington's disease, suggest that TG2 blockade might be promising in other protein aggregation conditions like Alzheimer's and Parkinson's diseases; 3. A new class of selective and

irreversible TG2 inhibitor, such as KCC009, was observed to enhance apoptosis of glioblastomas in a murine model; 4. Down regulation of TG2 expression in tumor cells hold great promise in reversing chemoresistance and inhibiting metastasis. Applying siRNA oligonucleotides for TG2 and using upcoming designed small molecule inhibitors, inhibiting specific TG2 functions mediated by distinct parts of the enzyme are several ways to counteract TG2 involvement in human pathology [9]. TG2 is necessary for the interaction between mast cells and macrophages during allergic inflammation and is responsible for the enhanced metastatic potential of tumor cells that are accompanied by allergic inflammation. A TG2 specific microRNA was suggested lately to attenuate the process progression [21]. TG2 inhibitors were suggested for ventilator-induced lung injury, pulmonary fibrosis and many kinds of cancerous conditions. Most

recently, a potent and specific dihydroisoxazole inhibitors of human transglutaminase2 was discovered, providing a clear basis for the rational selection of dihydroisoxazole inhibitors as tools for in vivo biological investigation [22].

It seems that we are at the verge of future approaches to design TG2 inhibitors that could be developed as potential disease-modifying therapies.

The expanded list of specific substrates for all the nine family members, open the window for a better understanding of the TG family functions [23]. The most recent potential role of TG2 in CD⁸⁺ T cell activation and CD⁸⁺ memory T cell generation might present an additional avenue for new therapeutic strategies [24].

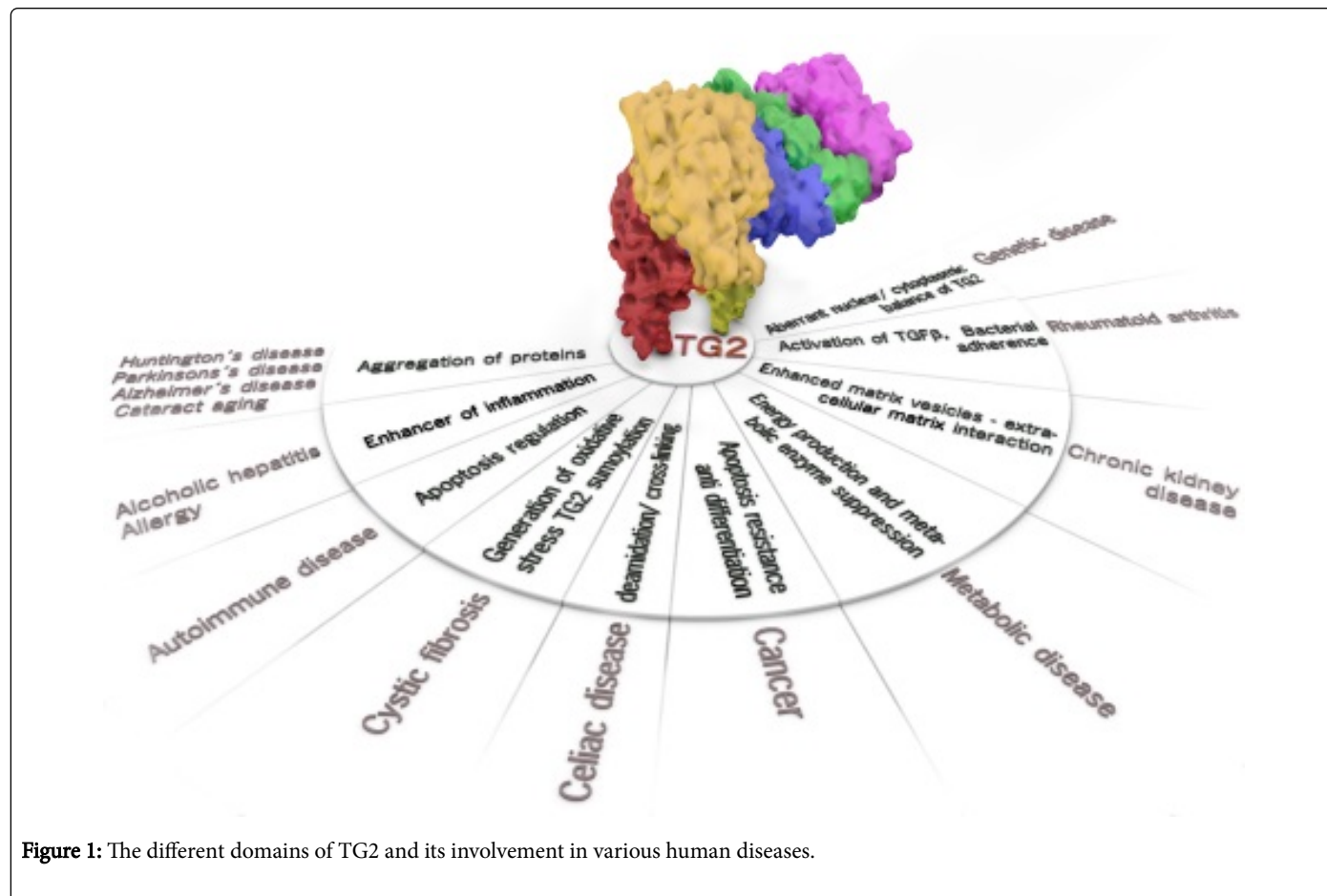


Figure 1: The different domains of TG2 and its involvement in various human diseases.

Conclusion

The abundance, its specialized structural confirmation, vast substrate specificity, and indispensable cellular functions, justify TG2 implication in myriads biological events. From the present review, it is evident that the extensive knowledge accumulated on the enzyme structure, functions and regulations, fertilizes the search for its importance in human pathological states. In reality, the enzyme plays a pivotal role in multiple human diseases spanning inflammatory, degenerative, neurodegenerative, malignant, metabolic, autoimmune and genetic conditions. As a consequence, designing specific TG2 inhibitors, to block its aberrant malfunction, is and will induce potential disease-modifying therapies.

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