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Tissue transglutaminase (TG2) and mitochondrial function and dysfunction

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1. ABSTRACT

Mitochondria are the cell's power plant to satisfy the energy demands. However, dysfunctional mitochondria can cause overproduction of reactive oxygen species (ROS), oxidative stress, and alteration of calcium homeostasis, which are the hallmarks of mitochondrial diseases. Under prolong oxidative stress, repeated cytosolic calcium elevations even only transiently, can lead to activation of some enzymes. One calcium-activated enzyme with demonstrated pathophysiological important in mitochondrial disease is tissue transglutaminase (TG2). TG2 is known as a post-translational modification (PTM) enzyme that is induced by oxidative stress. Compared to other types of PTMs, the physiological significance of TG2 mediated PTM is just beginning to be understood. Once activated, TG2 can modulate transcription, inactivate metabolic enzymes, and cause aggregation of critical proteins. Recent data indicate that TG2's activity not only can modulate the assembly of respiratory chain complexes but can also modulate the transcription of critical genes including PGC-1α and cytochrome C that are important for function and biogenesis of mitochondria. Here, we summarize dysfunctional mitochondria in diseases such as in neurodegenerative disorders can modulate TG2's activity and function. TG2 is also important for normal function of mitochondria.

2. INTRODUCTION

Mitochondria are the power plants of the cell and produce ATP to satisfy the cell's energy demands. The leakage of electrons from the mitochondria's electron respiratory chain and the generation of reactive oxygen species (ROS) were long referred to as a byproduct of oxidative phosphorylation and is one of the major sources of' intracellular ROS. Superoxide anion ($\cdot O_2^-$), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\cdot OH$) are the representative ROS in living cells. ROS are highly reactive oxygen-containing molecules that can oxidize and thus damage biologically important molecules such as proteins, fatty acids, and genomic DNA. Under normal conditions, ROS are removed by cellular antioxidant systems such as superoxide dismutase (SOD), catalase, glutathione, and thioredoxin. However, under pathological conditions with abnormal mitochondrial function, when the rate of ROS generation exceeds the limit of the cellular antioxidant system, oxidative stress occurs. Increased in oxidative stress is common in the development of diseases including cancer, cardiovascular, diabetes, autoimmune and neurodegenerative diseases (1). Damage from free radicals is also common in brains from patients with age-related neurodegenerative disorders. Also, a dysfunctional mitochondria is associated with abnormal cellular calcium levels, which could activate the intracellular calcium-dependent enzyme and cause further cellular injury (2).

Mitochondria play a critical role in the maintenance of intracellular calcium homeostasis and transmission of calcium signals. Under physiological condition, intracellular calcium level is tightly regulated by mitochondria via uptake and efflux of Ca^{2+} ions in cooperation with the endoplasmic reticulum (ER). In response to stimuli, Ca^{2+} ions released from ER induce a series of signal cascades, and this Ca^{2+} signals need to be interrupted by mitochondrial uptake and sequestration of Ca^{2+} ions in due course (3). Depending on the mitochondrial membrane potential and calcium transporters, the influx of Ca^{2+} ions into mitochondria boosts energy metabolism via directly activating several key metabolic enzymes and indirectly regulating metabolites transporters (4). On top of that, calcium buffering capacity enables mitochondria to modulate Ca^{2+} signaling that controls fundamental cellular processes (3, 5). However, excessive accumulation of mitochondrial Ca^{2+} is thought to cause mitochondrial depolarization and consequently trigger cell death (6). Massive Ca^{2+} influx attenuates the mitochondrial membrane potential leading to the neuron death that was demonstrated to be the primary event in glutamate neurotoxicity (7).

A large number of evidence manifests that mitochondrial calcium overload and subsequent dysfunction are involved in the pathogenesis of neurodegenerative disorders, mitochondrial diseases, and diabetes (5, 8). Excessive influx of Ca^{2+}-elicited mitochondrial dysfunction has been implicated in excitotoxic neuronal death in neurodegeneration and brain injury (8). Abnormal intracellular calcium also occurs in different models with mitochondrial electron transport chain defects (9, 10). Aberrant handling of mitochondrial Ca^{2+} ions was observed in primary skin fibroblasts from Myoclonus Epilepsy with Ragged-Red Fibers (MERRF) and Leigh's syndrome (11, 12). Abnormal calcium buffering of mitochondria and elevation of cytosolic Ca^{2+} ions has been reported in human cells harboring mitochondrial DNA (mtDNA)

mutation associated with MERRF and Mitochondrial myopathy, Encephalopathy, Lactic Acidosis, and Stroke (MELAS) syndrome (13, 14). A study showed that increased Ca^{2+} release from ER and consequent overload of cytosolic and mitochondrial Ca^{2+} occurs on respiratory enzyme Complex II-deficient fibroblasts caused by genetic mutation or inhibitor treatment (12). A similar phenomenon is observed in cells treated with mitochondrial ATP synthase inhibitor, oligomycin A. Not only that mitochondrial respiratory defects-evoked calcium dyshomeostasis could enhance the susceptibility to excitotoxicity in neuron via abnormal activation of Ca^{2+}-dependent protein kinase (15, 16). The aspects mentioned above indicate mitochondrial calcium homeostasis as a potential target for mitochondria-based pharmacological strategies.

Other examples of "mitochondrial diseases" are age-related diseases including Alzheimer's disease (AD) Parkinson's disease (PD) and Huntington's disease (HD) (1). In these disorders, distinct mitochondrial abnormalities culminate in oxidative stress, energy dysfunction, and aberrant homeostasis of cytosolic calcium (1). Under prolong oxidative stress, repeated cytosolic calcium elevations even only transiently, can lead to activation of some enzymes (1). One calcium-activated enzyme with demonstrated pathophysiological important in HD and AD is tissue transglutaminase (EC 2.3.2.1.3.; protein-glutamine γ-glutamyltransferase; designated as TG2). TG2 is known as a cross-linking enzyme that can modulate transcription, inactivate metabolic enzymes, and cause aggregation of critical proteins (17). Recently, TG2 was also found to display other functions (17, 18). These data indicate that TG2 can silence expression of genes involved in compensating for metabolic stress (1). Here, we summarize the role of mitochondria in modulating TG2's function in "mitochondrial diseases" such as neurodegenerative disorders and the role of TG2 in regulating mitochondrial function. TG2 is known as a post-translational modification (PTM) enzyme with transamidation and other activities (see below) (17, 18). Compared to phosphorylation, acetylation, and glycosylation in cell biology, the PTM mediated by TG2 is just beginning to be understood. Oxidative stress induces TG2 and TG2 is localized in many intracellular compartments including mitochondria. Abnormal of TG2 expression and activity is implicated in the development of various diseases related to aging (1, 17).

3. TG2: A MULTIFUNCTIONAL ENZYME

TG2 belongs to a family of closely related thiol enzymes called transglutaminases (TGs) that are derived from a common ancestral gene (17, 19, 20). At least eight enzymatically active TGs include blood coagulation factor XIII A-chains, TG1-7 and one inactive protein band 4.2. (17). Except for TG2, other TGs are expressed as zymogens and requires protease cleavage to become an active transglutaminase (17, 21, 22). Unlike other TGs, TG2 is unique in that it is ubiquitous and a multifunctional enzyme with Ca^{+2}-dependent transamidation activity (TGase), Mg^{+2}-dependent GTP/ATP binding and hydrolysis, and protein disulfide isomerase (PDI) activities with distinct substrate binding and enzyme catalytic domains (17, 19, 23, 24). Moreover, there are non-enzymatic functions of TG2 including to functioning as a cell-surface adhesion molecule, to bind NO, and to serve as a co-receptor for integrins and the G-protein coupled receptors (GPCR) including α_{β}-*adrenergic receptor (αAR) and GPR56.*

TG2 is also implicated in diverse biological functions including cell death, cytoskeleton rearrangement, gene regulation, and signaling function (25-28). TG2 is implicated in promoting programmed cell death, regulation inflammation, serving as a therapeutic target in neurodegeneration, fibrosis, autoimmunity, hypertension, Celiac disease and cardiovascular disorders (17).

3.1. Transamidation reaction (TGase function)

Depending on the pH and availability of the substrates, TG2 displays three types of transamidation reactions which can alter the solubility of the crosslinked protein substrates (18). We will describe these reactions in the following sections.

3.1.1. Inter- or intra-molecular crosslinking

TG2 catalyzes a Ca^{+2}-dependent crosslinking reaction between a peptide-bound γ-glutamyl (Q-substrate) residue (the acyl-donor) and a primary amine (the acyl-acceptor, K substrate). The most common primary amine acyl acceptors are the ε-amino groups of peptide-bound lysine *(*peptide-bound K-substrate*)* residues or primary amino groups of natural occurring biogenic amines or polyamines (free K-substrate)(Figure 1). The end product of the crosslinking reaction is the formation of a γ-glutamyl-ε-lysine (called isopeptide, a biomarker of crosslinking) bond between two proteins or between a protein and an amine. The isopeptide bonds can be either in the same protein (intra-) or between two proteins (inter-) (Figure 1A). At low substrate concentrations, the intra-molecular crosslinking reaction is preferred (29). The inter-molecular cross-linking reaction is kinetically favored at pH > 7 and higher substrate concentrations. The crosslinking reactions result in post-translational modifications of proteins that can alter their solubility, structure, and function (17, 21, 22).

Many Intra- and extracellular proteins have been identified as TG2 substrates (17). To date, there are at least 150 substrates reported on TRANSDAB

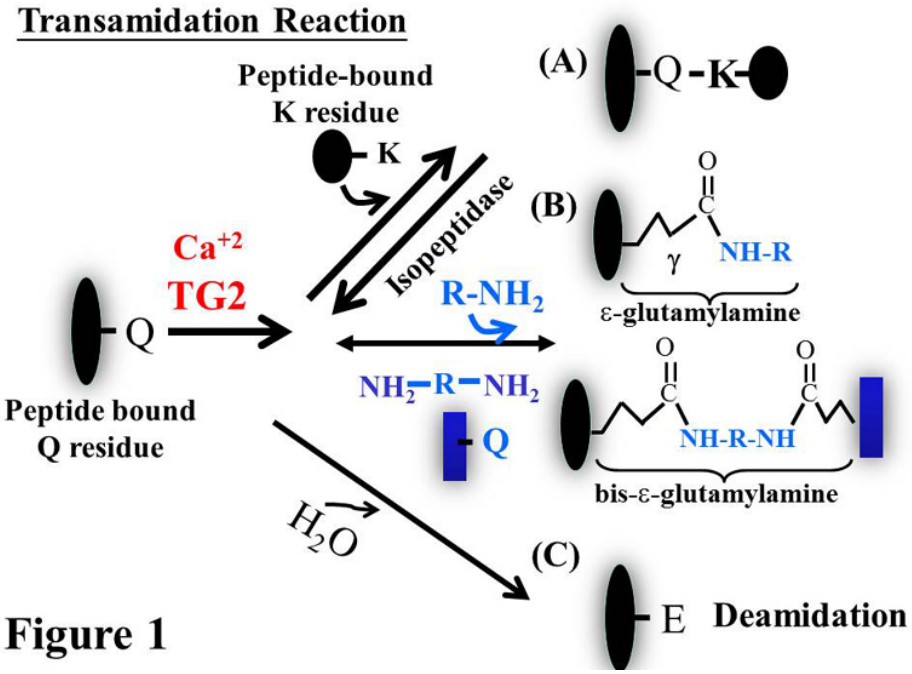

Figure 1. The Transamidation Reactions Catalyzed by TGase/TG2. In the presence of calcium, TG2 can catalyze three different types of transamidation reactions and the reaction products release ammonia (NH_3). (A). Inter- or intra-molecular crosslinking between Q- and K-containing peptides and forming a isopeptide bond; (B), Aminytion reaction between Q-peptide and primary amines (polyamines or biogenic amines). If a monmoamine (such as histamine) is involved, the reaction will form an -glutamylamine bond between protein and amine. If a diamine (such as putrescine) is involved in the crosslinking, the reaction will form a bis-ε-glutamylamine bond between proteins. (C). Deamidation reaction to convert peptide bound glutamine (Q) residue to glutamic acid (E) residue. The reversed reaction of A or B is catalyzed by the isopeptidase activity of TG2.

database (http://genomics.dote.hu/wiki/index.php/Category:Tissue_transglutaminase). There are considerably much more TG2's substrates identified in the database than other TGs, due either to more research on TG2 or ubiquitous nature of the enzyme. The intracellular protein substrates include K rich nuclear core histones, huntingtin, NF*k*B inhibitor alpha (IkBα) and mitochondria's key enzymes in glycolysis and TCA cycle including glyceraldehyde-3-phosphate dehydrogenase (G3PDH), α-ketoglutarate dehydrogenase (KGDHC) and aconitase (30-34). Cytoskeletal proteins including actin, tubulin, myosin and ROCK2 with roles in cell motility and adhesion are well defined TG2 substrates (21, 35). The G3PDH molecule was shown to be covalently bound to several proteins involved in neurodegeneration caused by polyQ expansion diseases (30, 36). Extracellular crosslinking is an important PTM of extracellular matrix (ECM) molecules for their mechanical and enzymatic stabilization. There are some ECM proteins including fibronectin (FN), collagens, osteopontin, nidogen/entactin, laminin, vitronectin and osteonectin that modulate matrix's structure and function (17, 19, 37).

3.1.2. Aminylation

Aminylation refers to the process by which primary amines (free K-substrate) including either biogenic/polyamines are covalently coupled to a peptide-bound glutamine residue(s) by TGs (18, 38-41). When TG2 is in the vicinity of a peptide-bound Q residue, and there are abundant primary amine substrates available (i.e., biogenic amines/polyamines), the enzyme catalyzes the incorporation of the primary amino group to glutamine resulting in the formation of a γ-glutamyl-amine bond (Figure 1B*)* (17, 18). If multiple glutamines are modified within the protein, this type of PTM is called polyaminylation, while modification of only one glutamine residue is called monoaminylation. The term, "serotonylation" or "histaminylation", are used to refer to when serotonin or histamine, respectively, are crosslinked to target proteins by a TG-mediated reaction (42-45). Comparing to Q substrates, TG2 shows less specificity toward K-substrates (46-48). TG2 prefers aliphatic amines with a chain equal in length to the side chain of a lysine residue (7.2.-7.6. Å) (48, 49). As synthetic primary amine inhibitors are relatively non-toxic to cells, they are widely used as intracellular inhibitors of TG by competing with natural intracellular amines (48). Cystamine is a non-specific and unique primary amine inhibitor known to inactivate the enzyme, probably by forming mixed disulfide bonds with TG2 (48).

Both biogenic amines and polyamines are highly charged low molecular weight aliphatic polycations. They are ubiquitously present in all living cells and implicated to play a role in a large number of cellular

processes (39, 42). Serotonin (5-hydroxytryptamine; 5-HT), histamine, catecholamines, noradrenaline, and dopamine are best studied biogenic amines (39, 42) that can function as TG2 substrates. Biogenic amines were demonstrated to be covalently incorporated into proteins involved cell signaling, inflammation and other vascular biological processes (38-42). Common polyamines include *putrescine* (a diamine), *spermidine* (a triamine) and *spermine* (a tetramine). Intracellular polyamines play an important role in regulating different cellular processes including cell growth, apoptosis, and differentiation and their levels are tightly controlled (50). Diamines such as putrescine, the other free amine group can be further crosslinked to another glutaminyl moiety, forming a bis-γ-glutamylpolyamine bond (Figure 1B) thereby catalyzing inter or intra-molecular covalent bonds. When covalently modified by biogenic amines/polyamines, the surface charge of the target proteins can be altered and potentially alter protein-protein interactions. In addition, proteins' solubility can be altered by aminylation. Expanded polyQ containing proteins (such as Huntingtin in Huntingtin's disease; HD) and tubulins (in neuron's neurite formation) crosslinking either through protein-protein or protein-amine can dramatically change these protein's solubility and function (51, 52).

Histamine was found to be the most effective biogenic amine inhibitor *in vitro* with an IC_{50} value of 160 μM followed by putrescine (IC_{50} ~ 600 μM), whereas > 50 mM of dopamine and serotonin only inhibited < 10% of TGase/TG2 (40). Putrescine and histamine were also demonstrated to be better substrates than spermine, spermidine, and serotonin (5-hydroxytryptamine) (53). Although serotonin is not a good substrate of TG2, it is highly concentrated in platelet dense bodies and the concentration is reported to be up to 65 mM (38, 42). Therefore, local environmental and cofactors may be needed for serotonin or other biogenic amines to become TG2 substrates.

Aminylation of histone 3 (H3) N-terminal tail was shown to downregulate the transcription of target genes, peroxisome proliferator-activated receptor-γ coactivator 1a (PGC-1α) and downstream cytochrome C, genes that are important for biogenesis of mitochondria (54), contributing to dysfunctional mitochondria in HD (55). Not only is H3 but H2A, H2B and H4 were all shown as TG2's substrates (33).

TG2 levels and free isopeptide (γ-glutamyl-ε-lysine) levels isolated from patient's cerebrospinal fluid (CSF) are significantly elevated in HD patients indicating that TG2 cross-linking is an active process in HD (56, 57). Detailed HPLC analysis of samples from human CSF demonstrated that γ-glutamyl-ε-lysine, γ-glutamylspermidine, γ-glutamylputrescine, and bis-γ-glutamylputrescine are all present and all are present at higher levels in HD brain (58, 59). These data indicate that TG-mediated aminylation reaction is an active process during HD. The elevated free isopeptide levels in samples derived from CSF represent the degradation products of soluble crosslinked aggregates in HD patients (56), which is consistent with the emerging hypothesis that soluble cross-linked aggregates are neurotoxic**.**

In summary, as the TG-mediated isopeptide peptide bonds are protease resistant, the above two crosslinking reactions may potentially contribute to several pathologic processes involved in neurodegeneration including neuroinflammation, accumulation of insoluble protein inclusions, and proteasome dysfunction (60). TG2 is also known to catalyze both inter- and intra-molecular crosslinking of tau protein, α-synuclein (SYN), and huntingtin forming soluble oligomers and insoluble aggregates (29, 61, 62). There is an emerging hypothesis that soluble and diffusible high molecular weight oligomeric complexes (or micro-aggregates) are the neurotoxic intermediates in neurodegeneration, while the insoluble inclusions represent a non-toxic pool of insoluble proteins (63, 64). TG2 mediated intermolecular crosslinking of polyQ containing proteins (i.e., mutant huntingtin in HD or other expanded polyQ diseases) may lead to the formation of soluble protein intermediates and slows the formation of insoluble protein aggregate (51).

3.1.3. Deamidation

When the concentrations of peptide-bound K substrate and 1° amine are lower than the K_M and the reaction pH < 7, water can act as the acyl-acceptor. The resultant hydrolysis reaction converts glutamine (Q) to a glutamic acid (E) residue (*deamidation)* (Figure 1C)(17, 21, 22, 32). In Celiac disease patients, specific deamidation of a select glutamine residue in gluten peptides leads to the development of an autoantibody against TG2 (18, 21). Clinically, anti-TG2 IgA autoantibody serves as a diagnostic marker for celiac disease and assist in monitoring celiac disease (17). Hsp20 was shown to be deamidated at a specific glutamine residue while distinct glutamines were substrates in other types of crosslinking reactions (65). G3PDH was found to be deamidated by TG2 at multiple glutamine residues and deamidated G3PDH promoted trophoblastic cell fusion (66). These results suggest deamidation may be a more widely occurring event than previously recognized.

3.2. Isopeptidase activity

Although isopeptide bonds are considered resistant to proteolytic digestion, TG2 can reverse the transamidation reaction and hydrolyze specific isopepeptide bonds (53, 67, 68). TG2's isopeptidase activity is a calcium-dependent reaction (68). When putrescine and histamine were covalently crosslinked

to gluten-derived peptide, TG2 was more efficient in hydrolyzing histamine-gliadin than putrescine-gliadin peptides. These data suggest that there is a preference for primary amines in hydrolyzing the isopeptide bonds (53). Due to the difficulty in detecting the hydrolyzed product, the physiological importance of isopeptidase reactions remains poorly defined but demonstrates the dynamic nature of the TGase-catalyzed crosslinking reactions.

3.3. Protein Disulfide Isomerase (PDI) activity

PDI can catalyze the formation, breakup, and exchange of disulfide bonds using reactive cysteine residues (23). The PDI activity is typically localized in the ER and on the surface of eukaryotic cells. TG2's PDI activity requires free thiol cysteines and therefore is sensitive to cellular levels of oxidants/antioxidants. TG2 was found to have relatively low but detectable PDI activity. Unlike TGase activity, the PDI activity is independent of Ca^{+2} and GTP (23). The activity is increased by oxidized glutathione but inhibited by reduced glutathione (23). Interestingly, PDI-related protein in filarial parasite also possesses TGase activity (69). Several PDIs and related thioredoxins were found to display TGase activity and have the same conserved Cys-His–Asp triad residues as TGase active site center found in all TGs (70). These data suggest that PDIs, thioredoxins and TGs share some overlapping function in cell and tissue. TG2's PDI is important in the assembly of ADP/ATP transporter in mitochondria and will be discussed in the following section (71, 72).

3.4. GTP/ATP hydrolysis activity

In the presence of Mg^{+2}, TG2 can hydrolyze Mg-GTP and Mg-ATP at a similar rate. TG2 interacts with Mg-ATP (K_M = 38 +/- 10 microM) at a 3-fold greater steady-state affinity than with Mg-GTP (K_M = 130 +/- 35 microM). Also, Mg-ATP inhibited GTP hydrolysis (IC_{50} = 24 microM), whereas 1 mM Mg-GTP reduced ATP hydrolysis by only 20% (73). G protein ($G_{\alpha h}$) function of TG2 can control β-adrenergic-receptor-mediated signaling transduction pathways that lead to PLCδ1 activation (17, 22, 27). PLCδ1 activation results in an increase in intracellular calcium which activates the transamidation reaction (17). GTP induces a conformational change that inhibits TGase/TG2's activity by narrowing the active site pocket, a process that can be reversed by high concentration of Ca^{+2} (17, 22). The ATP hydrolysis function of TG2 remains poorly understood.

4. Structure and function of TG2

4.1 TGase active site.

TG2 is a protein with 687 amino acid residues. X-ray crystallography reveals that TG2 is composed of an N-terminal β-sandwich (residue #1-139; Domain I), a α/β catalytic core (residue # 140-454; Domain II), a β-barrel 1 (residue # 479-585; Domain III) and a β-barrel 2 (residue # 586-687; Domain IV)(74, 75). The TGase/TG2 active site is composed of a catalytic triad of C^{277}-H^{335}-D^{358} (74), and the rate-limiting step in catalysis involves the formation of a transitional thioester bond between C^{277} and the Q substrate. Based on the recent inhibitor-bound crystal structure studies, there are open catalytic active conformation, and open catalytic inactive conformation (21, 75).

4.2 GTP and ATP binding site

Based on the 3-D structure of GDP-bound TG2 (PDB: 1kv3), GTP binding involves the amino acid side chains from domains II, III and IV (74). The 3-D structure of ATP-bound TG2 also demonstrates that ATP and GDP bind to the same nucleotide binding pocket (82). However, S482 and R580 were found to be involved only in guanine, not adenine binding (80). Mutation at R580 to adenine resulted in almost complete loss of GTP/GDP activity as demonstrated by GTP binding assay but remained active in TGase function (21) and GTP hydrolysis (unpublished observation).

5. REGULATION OF *IN VIVO* TGase ACTIVITY BY GTP, REDOX, AND NITRIC OXIDE (NO)

5.1 Regulation of in vivo TGase activity by GTP.

The intracellular free Ca^{+2} (sub-microM) and GTP (~ 100-150 μM) are sufficient to keep the TGase/TG2 in a latent state (17, 21, 32). The activation of intracellular TGase/TG2 activity is a tightly controlled but poorly understood process that can be either beneficial or detrimental to cells. When cells are exposed to biotinylated pentylamine (BP, a synthetic 1° amine) or spermine, several intracellular proteins were biotinylated and this method has been used to identify TGase/TG2 substrates (76, 77). Using biotinylated 1° amines as probes, physiological levels of histamine, or serotonin were also found to be incorporated into cellular proteins under normal cell culture growth conditions (41, 53, 78). These data suggested that changes in intracellular Ca^{+2} concentrations or other cofactors are sufficient to activate intracellular TGase/TG2's activity. In diseases such as HD, it is possible that, TG2 becomes activated by repeated, oxidative stress mediated by expanded polyQ proteins and small transient rises of Ca^{2+} concentration (79), allowing for the gradual accumulation of soluble and/or insoluble cross-linked products over a long time period which would provide further neurotoxic crosslinked products. This finding may further explain why these diseases manifest so late in life.

5.2. Regulation of *in vivo* TGase activity by redox

The Intra- and extracellular redox state also regulate the activation of TGase/TG2. Because of high levels of calcium ions (~ mM) in the extracellular space, it is believed that TGase/TG2 is constitutively active (17, 19). However, it was reported that TGase/TG2 is not active due to oxidation and thioredoxin is involved in the formation of an intra-chain disulfide bond (80, 81). Three cysteine residues including C230, C370 and C371, were found to have high redox potential (81). Mutation analysis identified C230 as the key redox sensor (81). Due to extracellular oxidizing environments, the formation of intramolecular disulfide bonds (between C370-C371 and between C230-C370) are found to inactivate the TGase/TG2 (80). In the ATP-bound form of TG2, disulfide bond was formed between C230-C370 and may also contribute to its conformation under oxidizing conditions (82). Therefore, the activation of extracellular TG2's activity is also controlled by the redox potential of the local environment and availability of thioredoxin.

5.3. Regulation of *in vivo* TGase/TG2 by NO

Under reducing environment, TG2 has 18 free sulfhydryls (-SH), several of which are contained within S-nitrosylation motifs (25, 83). S-nitrosylation of TG2 by NO was also found to inhibit TGase/TG2 activity *in vitro* (25, 84). TG2 was found S-nitrosylated in a young aorta but not in an aged aorta indicating there was more TGase/TG2 activity in aged aorta suggesting NO can modulate TG2 activity *in vivo* (84).

In summary, local Ca^{+2}, GTP and redox potential are important factors in modulating the *in vivo* TGase/TG2. Under reducing environments, physiological levels of GTP inhibit TGase, while sufficient Ca^{+2} can reverse the GTP inhibition (73). In the oxidizing environments, thiol reductases such as thioredoxin might be the additional factor in controlling *in vivo* TGase activity of TG2 (81).

6. REGULATION OF TG2 EXPRESSION

The promoter of TG2 gene contains important response elements including retinoic acid (RA) (retinoic acid response element; RRE-1 and RRE-2), glucocorticoid (GRE), NFkB (85). In addition, the response elements for IL-6, TGFβ1, activator protein-2 (AP-2), hypoxia (HRE), and activator protein-1 (AP-1) are also present(17, 21, 32). Many of these molecules play a role in cellular responses to tissue injury. Among these inducers, the cell differentiation agent RA was the first agent identified to induce TG2 (86). Within 6 hours after the addition of RA, TG2 was found to increase by at least 50-fold (86). Soon after RA treatment, there is a redistribution of TG2 from cytoplasm to the plasma membrane (87). The significance of redistribution of TG2 after RA treatment remains to be investigated. We will summarize some of the major regulators of TG2 in the following section. Among these regulators, NF*k*B and HIF1 are two of the ROS-sensitive transcription factors that regulate the expression of TG2.

6.1. NF*k*B regulates the expression of TG2

Increased expression of TG2 and its TGase activity is a common feature of increased ROS and several inflammatory disorders (17, 22). An important transcription factor that is induced by ROS and inflammation is NF*k*B. TG2 is involved in enhanced inflammation by participating in an inflammatory loop with the "master switch" for inflammation involving NF*k*B. The TG2 gene promoter contains a NF*k*B response element (85). TG2 can also activate NF*k*B by cross-linking and polymerize I*k*Bα, the inhibitor of NF*k*B. Intracellular TG2 crosslinking events promote inflammation by activating the noncanonical pathway of NF*k*B (88, 89). Under normal condition, NF*k*B is inactive due to tight association with IkBα. However, in inflammatory conditions, IkBα is phosphorylated by IkB kinase (IKK), causing proteasome-dependent degradation of IkBα and releasing of its inhibition on NFkappaB. Free NF*k*B is then translocated to the nucleus to activate transcription of several important downstream inflammatory genes. In IKK independent pathway, IkBα is polymerized by TGase/TG2 results in its degradation by proteasome leading to NF*k*B activation (34). In another mechanism, TG2 can interact directly with IkBα leading to its degradation via a proteasome-independent pathway (90, 91). These data indicate that targeting TG2 may block IKK-independent pathway for the activation of NF*k*B.

6.2. Hypoxia regulates the expression of TG2

There are six putative hypoxia response elements in the promoter region of the TG2 gene (92). TG2 is the transcription target of HIF1, a heterodimeric transcription factor consisting of inducible HIF1α and constitutively expressed HIF1β, during the survival of neurons exposed to oxygen and glucose deprivation (93). During oxygen and glucose deprivation, TG2 protects against hypoxia by interacting with HIF1β and attenuates the HIF1 signaling (93). Under ischemia and stroke, TG2 may play an important role in protecting against the delayed neuronal cell death (93).

6.3. TGFβ regulates the expression of TG2

There is a TGFβ response element upstream of the transcription start site of TG2 gene (92). Bone morphogenetic factor 2 (BMP2) and 4 (BMP4) also regulate the TG2 expression by binding to the TGFβ response element (92). The up-regulation of TG2 by TGFβ1 leads to another positive feedback loop. TG2 is also involved in the conversion of latent to active

TGF-β1 and the TG2 itself are induced by TGF-β1 (94). TGFβ expression downregulates inflammatory and autoimmune responses (95) demonstrating that TG2 is involved in these complex biologic and pathologic processes. $TG2^{-/-}$ mice fail to activate TGFβ, have delayed clearance of apoptotic cells and had evidence of tissue inflammation and autoimmunity (96). The co-expression of the TG2 and active TGF-β1 at sites within wounded regions and sites of inflammation suggested a role in the wound healing response (97-99). Using a dorsal skin flap window chamber in vivo animal model, the direct application of recombinant TG2 to a mammary adenocarcinoma resulted in an increased level of collagen around the tumor and a fibrotic response (97). These data demonstrate a role of TG2 in the wound healing response and abnormal TG2's activity could lead to tissue fibrosis.

6.4. Oxidative stress and EGF regulate the expression of TG2

Oxidative stress appears important in glutamate-evoked TG2 upregulation in astrocyte culture (100). It is known glutamate causes a dose-dependent increase in ROS production. However, pre-incubation with a TG2 inhibitor, cysteamine, recovers oxidative stress and reduces glutamate-increased TG2 (100). Thus, TG2 up-regulation may be part of the biochemical responses to oxidative stress (100). TG2 is also induced by epidermal growth factor (EGF), inflammatory cytokines (IL-6, TNFα, IFNγ) and by various stimuli such as UV light, and viral infection (17, 101-103). Alterations in TG2 activity and function have been linked to cancer and other chronic diseases including atherosclerosis (17, 22). Thus, the induction of the TG2 expression and activation of its activity are associated with tissues response to various stimuli that lead to cell differentiation, inflammation, fibrosis and immune defense.

7. TG2 is LOCALIZED in MITOCHONDRIA and SEVERAL OTHER LOCATIONS

TG2 is a nuclear-encoded protein. The expression of TG2 is considered ubiquitous, but the distribution and expression levels vary significantly among different cell types. TG2 is expressed in cells involved in immunity and inflammation including lymphocytes, PMNs and monocytes (104, 105). Upon differentiation into macrophage, TG2 is up-regulated dramatically (106). The highest TG2 expression is found in vascular endothelial and smooth muscle cells (VSMC) (101, 105). TG2 is localized at the extracellular space, membrane-associated, cytoplasm, mitochondria and nucleus of the cell (17, 21). In human neuroblastoma cells, 7% of the total TG2 is found in the nucleus in association with chromatin (107). The significance of TG2 induction associated with intracellular translocation to the nucleus and change in intracellular TGase activity is poorly understood (107, 108). The cell surface TG2 can function as a cell adhesion molecule that interacts with leukocytes (109), a wide variety of extracellular matrix (ECM) adhesion proteins including integrins fibronectin, and GPR56 to increase the adhesive property of cells (110). The membrane-associated TG2 is reported to interact with GPR56 and IGFBP-3 (insulin growth factor binding protein) kinase in breast cancer cells and involved in G-protein and ATP kinase signaling function (111-113).

Although there is no classical mitochondrial targeting signal, TG2 is found in mitochondria in various cell types; in neuroblastoma cells, it constitutes up to 50% of the total TG2 cellular pool (114, 115). Using cellular fractionation and electron microscopy to investigate the localization, the majority of TG2 was found to localize to the mitochondrial outer membrane and the inner membrane space, while 5-10% of the protein is localized in the inner mitochondrial membrane and the mitochondrial matrix (114, 116). The mechanism of how TG2 is localized to specific cellular compartments is poorly understood as it does not have a secretion recognition signal, membrane anchor, or nuclear localization sequences. Through unknown mechanisms, TG2 mediates the expression of the $gp91^{phos}$ subunit of NADPH oxidase expression in PMNs, a gene product that is essential for oxidative killing through the generation of superoxide anions (117).

8. TG2 AND MITOCHONDRIAL FUNCTION

The role of TG2 in mitochondrial function is emerging. Earlier evidence support that TG2 play a role in energy metabolism came from the observations that the heart of TG -/- mice appeared to be more sensitive to ischemia/reperfusion injury (118). TG2 was postulated to participate in mitochondria's respiratory function, as TG -/- mice had a serious defect in ATP production (118). Also, the phenotype of TG-/- mice resembled that of maturity-onset diabetes of the young (MODY), suggesting a role of TG2 in mitochondrial functions outside cardiac muscle (119). Additional studies indicate that TGase/TG2 and PDI function are involved in mitochondrial functions as described below.

8.1. Activation of TGase/TG2 occurs under stress conditions

It is believed that TGase/TG2 mediated covalent crosslinking of mitochondrial proteins does not occur in normal tissues; however, TGase/TG2 is likely to be activated in "mitochondrial diseases" patients, including cardiovascular ischemia/reperfusion injury and neurodegenerative disorders (such as HD). That is due to abnormal calcium levels associated with mitochondrial dysfunction in these diseases. There

Table 1. Proteins interacting with TG2 important for mitochondrial functions

Proteins	Function	TG2 activity involved	References
G3PDH	TCA Cycle	TGase	30
KGDHC	TCA Cycle	TGase	30
Aconitase 2	TCA Cycle	TGase	123
Bax	Apoptosis	TGase	115
Prohibitin	Respiratory Chain	PDI	120, 121
ATP Synthase β	Respiratory Chain	PDI	120, 121
ANT1	ADP/ATP exchange	PDI	72

are consensus sequences ^{204}LKNAGRDC^{211} in TG2 which is 70% homologous to the BH3 domain of Bcl-2 family proteins, suggesting that it is a novel apoptotic BH3 protein (115). When apoptosis was induced by staurosporin, the proapoptotic protein Bax was found to interact with TG2 and was the major TGase /TG2 substrate during apoptosis (115). These data provide further support a role of TG2 in apoptosis.

A number of TGase/TG2's substrates were identified upon induction of intrinsic apoptosis pathway with staurosporin. These include prohibitin, Hsp70, Hsp90, Hsp60, and ATP synthase β chain. The correct folding of the Hsp70 and Hsp90 respiratory chain components require prohibitin which is a membrane-bound chaperone. In cooperating with prohibitin, the Hsp60 protein forms a membrane-tethered import motor complex involved in the unfolding of preprotein domains, whereas the ATP synthase β chain is a key component of complex V of the respiratory chain. Upon triggering mitochondria-dependent apoptosis in neural cells, these proteins were crosslinked by TGase/TG2 (120, 121). A similar crosslinking reaction also occurred *in vitro* and *in situ* with the TG2-binding partner, the bifunctional adenine nucleotide translocator (ANT1), a protein involved in ADP/ATP exchange and a core component of the permeability transition pore complex in the internal mitochondrial membrane (72). These data indicate that TGase/TG2 can be activated under stress condition (such as in apoptosis) to crosslink important proteins that are involved in the assembly of the respiratory chain.

8.2. TG2 and energy metabolism

Activation of TGase/TG2 under stress condition may be a possible cause of the decline in energy metabolism in neurodegenerative disorders (1). Although TG2 is not directly involved in metabolic function, it can post-translationally modify critical metabolic enzymes (Table I). Previous studies have shown that several important metabolic enzymes including fructose-1,6-bisphosphate aldolase, aconitase, L-lactate dehydrogenase (LDH), G3PDH, alpha-ketoglutarate dehydrogenase (KGDHC), phospho-glycerate dehydrogenase, fatty acid synthase, and aldehyde dehydrogenase, are functioning as TGase/TG2 substrates (30, 121-124)(Table I). Several of which are key enzymes of glycolysis and the TCA cycle, but the exact metabolic alteration of these enzymes after modification by TG2 have not been investigated in details. Two enzymes, G3PDH and KGDHC, were previously shown to be crosslinked and inactivated by TG2 in a cellular model of HD (30). TG2 also interact with pyruvate kinase M2 (PKM2). PKM2 is a rate limiting enzyme of glycolysis which is responsible for maintaining a glycolytic phenotype in malignant cells (122). Interaction of PKM2 and TG2 also plays an important role in the regulation of autophagy in particular under stressful conditions such as those displayed by cancer cells (122). All these findings suggest that modification by TG2 or interaction with TG2 may modulate and regulate the energy homeostasis and this warrants further investigation.

8.3. TG2 and calcium homeostasis

As calcium ions regulate an enormous number of cellular processes, the intracellular calcium levels are under very tight control. Both mitochondria and ER are intracellular stores for Ca^{+2} and 5-20% of the mitochondrial membrane surfaces are connected to the ER membrane domains called mitochondria-associated membranes (125). Although TG2 is activated by calcium under stress conditions, it also plays a role in regulating intracellular calcium homeostasis (126). Inositol 1,4,5-triphosphate receptors (IP3Rs) are ligand-gated ion channels that regulate the release Ca^{+2} from the ER (127, 128). IP_3Rs are allosteric proteins comprising four subunits that are assembled into a calcium channel, which normally opens by changing the spatial relationship between the four subunits (127, 128). In HD mice model, up-regulation and activation of TGase/TG2 results in the crosslinking of Q2476 of IP3R to the adjacent subunit's protein bound K-residue that lock the channel into an irreversible configuration (126). As reversible and repetitive structural changes are required for ligand-gated ion channels to mediate biological signaling, TG2 crosslinking chronically impaired calcium signaling and autophagy regulation in living cells (126).

Upon stimulation of rat insulinoma cell line (INS-1E) with glucose, many mitochondrial proteins involved in Ca^{+2} homeostasis including voltage-dependent anion-selective channel (VDAC) protein, prohibitin, and different ATP synthase subunits were found to be the TGase substrates of TG2 (129). VDACs are part of a network which includes the IP3Rs, stress-70 protein, a mitochondrial chaperone that facilitate mitochondrial Ca^{+2} uptake, and calreticulin (129).

In another study when overexpressed TG2 in Jurkat T cells, part of the TG2 was found to co-localize with mitochondria and enhanced mitochondrial Ca^{+2} uptake (130). Increased mitochondrial Ca^{+2} uptake was associated with the initiation of apoptosis (130). Overexpressed TG2 was found to crosslink RAP1, GTP-GDP dissociation stimulator 1, an unusual guanine exchange factor acting on various GTPase (131), which appeared in the ER to induce a yet uncharacterized signaling pathway that was able to promote Ca^{+2} release from the ER (130).

The above studies indicate a potential role of TG2 in regulating calcium homeostasis.

8.4. TG2 in modulating transcription of important mitochondrial genes

TGase/TG2 can also affect mitochondrial function by acting at the transcriptional level under pathological conditions such as in HD (54, 132). In HD mouse model which overexpress mutant huntingtin, activation of TGase/TG2 activity results in polyamination and adding positive charges to histones 3 (H3) N-terminal tail and leads to tighter packing of DNA with histones (54). As a result of the PTM, the transcription of target genes, peroxisome proliferator-activated receptor-γ (PPARγ), coactivator 1α (PGC-1α) and downstream cytochrome C (Cyt C), genes that are important for biogenesis and function of mitochondria are downregulated (54). The resulting consequences were postulated to contribute to the observed dysfunctional mitochondria in HD (55). In contrast, TG2 -/- mice as well as pharmacological inhibition of TG2 induce cytochrome C promoter activity and increase mRNA levels of Cyt C and PGC-1α. Citrate synthase activity**,** the first step of the TCA cycle, also increase after TG2 inhibition (54). These data suggest that TG2 may be a therapeutic target in mitochondrial diseases such as in HD and AD. Whether the activation of TGase/TG2 in regulating transcription factors is a physiological regulatory mechanism or only in the presence of mutant huntingtin warrant further investigation.

TG2 can also alter transcription through post-translational modification of histone proteins. Not only is H3 a substrate but H2A, H2B and H4 were all TG2 substrates (33). TG2 is involved in altering the proteins that play a role in regulating transcription and the specific genes and their impact on cells remains to be determined.

8.5. TG2's PDI activity is important in the assembly of respiratory chain complexes

TG-/- mice had energy production impairment as evidenced by decreased ATP levels after physical challenge (71). PDI activity was postulated to contribute to the correct assembly of the respiratory chain complexes. TG2-/- mice were found to have dysfunctional disulfide bond formation in complex I (NADH-ubiquinone oxidoreductase), complex II (succinate–ubiquinone oxidoreductase), complex IV (cytochrome C oxidase), and complex V (ATP synthase) (71).

Another target of TG2's PDI activity is ADP/ATP transporter adenine nucleotide translocator 1 (ANT1). ANT1 is the most abundant mitochondrial protein and a bi-functional protein primarily involved in ADP/ATP exchange. ANT1 is essential for normal mitochondrial function and has also been proposed to be among the various components of the permeability transition pore (PTP) in the inner mitochondrial membrane (133). ANT1 oligomerization is essential for its activity and TG2's PDI activity regulates the ADP/ATP transporter function by controlling the oligomerization of ANT1 (72). Increased thiol-dependent ANT1 oligomer formation and elevated ANT1 ADP/ATP exchange activity in heart mitochondria were found in *TG2*$^{-/-}$ mice (72). Therefore, the PDI/TG2 activity reduces the level of oligomerized ANT1. The PDI/G2 also inhibit transporter activity by sequestering ANT1 monomers and preventing oligomer formation by direct binding to ANT1 (72). These data reveal an important role for PDI/TG2 activity *in vivo* and indicate that there is a novel pathway that links this activity with the regulation of mitochondrial physiology.

8.6. TG2 and Warburg effect

Using embryonic fibroblasts (MEFs) derived from TG2 -/- mice, deletion of TG2 leads to mitophagy (clearance of defective mitochondria) impairment associated with a metabolic shift towards aerobic glycolysis (134). MEF cells (from TG2 -/-) showed increased oxygen consumption rate (OCR) (134). The function of mitophagy is to remove dysfunctional mitochondria to alleviate oxidative stress and prevent carcinogenesis (135). MEFs derived from TG2-/- mice display fragmented mitochondria with altered morphology and depolarization of the mitochondrial membrane (134). MEFs (from TG2-/- mice) also showed reduced ROS formation and down-regulation of the $GP91^{phox}$ (117). These studies indicated a role of TG2 in contributing to dysfunctional mitochondria.

In breast cancer epithelial cells, TG2 was shown to be an important regulator of the Warburg effect (136). Normal differentiated cells rely primarily on mitochondrial oxidative phosphorylation to generate the energy needed for cellular processes, while most cancer cells instead rely on aerobic glycolysis, a phenomenon termed "the Warburg effect." (137) High TG2 expressing breast cancer cells display a decrease in oxygen consumption rate (OCR) accompanied by an increase in extracellular acidification rate (ECAR) even under normoxic conditions (136). Knockdown TG2 by siRNA reverses the process. TG2/NFκB-induced increase in HIF-1α expression was associated with increased glucose uptake, increased lactate production and decreased oxygen consumption by mitochondria (136). In these tumor cells, low energy production by this glycolytic pathway is compensated by increased glucose uptake. Also, HIF-1α downregulates oxidative phosphorylation (91). The data is consistent with renal carcinoma cells (RCC) in that over-expression of TG2 increased glucose consumption and lactate production, while TG2 siRNA decreased lactate levels by 20-30% (124). These studies indicated overexpression of TG2 shifts tumor cells to more aerobic glycolysis pathway.

9. TG2 IN VARIOUS DISEASES AND BIOLOGICAL PROCESSES

Beside the role of TG2 in modulating mitochondrial function, TG2 is demonstrated to play a role in diseases and biological processes related to inflammation as described below. Among these, the role of TG2 in neurodegenerative diseases has been well studied (17, 21). In neurodegenerative diseases, impaired mitochondrial function is common and results in activation of TGase/TG2 (17, 21). It would be interested to examine the activation of TGase/TG2 in other mitochondrial diseases including MELAS, MERRF, and CPEO syndromes which are due to specific point mutations or large deletions of mitochondrial DNA (2, 138).

9.1. Epithelial-Mesenchymal Transition (EMT)

EMT is a critical process in cancer progression (139). EMT is characterized by breakdown of cell junctions and loss of cell polarity, rendering epithelial cells motile and invasive (139). TG2 positively influences the development of EMT through at least two mechanisms. First, it cross-links the large latent form of TGFβ to the extracellular matrix (140), this may concentrate or release bioactive TGFβ, an inducer of EMT. TG2 and TGFβ reciprocally induce each other as part of an auto-stimulatory loop, thus emphasizing the role of TG2 in the EMT process. Second, TG2 activates NF*k*B, a recognized EMT inducer, by cross-linking and polymerizing the inhibitor of NF*k*B, I*k*Bα, leading to its proteasomal degradation (90). Inhibition of TG2 expression by siRNA blocks EMT induction (141). The role of TG2 during EMT process has also been studied in several other different cell types (21).

9.2. Autoimmunity

Intracellularly, TGase/TG2 activity is important in forming apoptotic body preventing the release of intracellular proinflammatory substances. Apoptotic bodies also have an anti-inflammatory effects by inducing the anti-inflammation cytokine TGF-β (142). $TG2^{-/-}$ mice have abnormalities in clearing apoptotic cells and cause immune dysfunction and inflammation (143). $TG2^{-/-}$ mice fail to activate TGFβ, have delayed clearance of apoptotic cells and have evidence of autoimmunity (96).

9.3. Neurodegenerative diseases

TGase-mediated crosslinking are postulated to contribute to several pathologic hallmarks in neurodegenerative diseases including neuroinflammation, accumulation of insoluble protein inclusions, and proteasome dysfunction (60). Although intracellular TGase activity is tightly regulated, it is possible that, TGase/TG2 becomes activated by repeated responses from intracellular injuries including oxidative stress mediated by mis-folded proteins such as expanded polyQ proteins and results in small transient rises of Ca^{2+} concentration (79).

9.4. Huntington's disease (HD)

TG2 is known to catalyze the inter- or intra-molecular crosslinking of tau protein, α-synuclein (SYN), and huntingtin forming soluble oligomers, while unmodified or polyaminated disease proteins produced insoluble inclusions (29, 61, 62). *In vivo* data have validated TG2 as a target for inhibition in HD. Data from cross-breeding TG2 knock-out (TG2 KO) (TG2 -/-) and two different models of HD (R6/1 and R6/2) mice (see below)(144, 145) and pharmacological (cystamine) inhibition (146, 147) all show a beneficial effect of inhibiting TGase/TG2.

9.5. Parkinson's disease (PD)

The following important findings demonstrate a role of TG2 in Parkinson's disease (PD): 1) The discovery of synuclein (SYN) protein as an *in vitro* and *in vivo* TG2 substrate (148, 149); 2) the increased levels of TG2-catalyzed isopeptides co-localized with SYN in Lewy bodies and were correlated with the severity of PD patients (149). TG2 was found to interact directly with SYN both *in vitro* and in cell models (148, 150). Andringa *et al*. demonstrated that SYN is a TGase/TG2's substrate *in vivo (149)*. Increased levels of TG2-induced intra- and intermolecular cross-linked SYN were found in PD brains, suggesting that this cross-link

precedes further aggregation of SYN into Lewy bodies (149). Also, crosslinked products of ubiquitin, hsp27, and SYN were also demonstrated in Alzheimer's brains (151). As the crosslinked products are protease resistant isopeptide bonds, they were postulated to interfere with ubiquitin-proteasome degradation of misfolded proteins (151).

9.6. Alzheimers' Disease (AD)

The hallmarks of AD are the formation of extracellular neurotoxic aggregates consisting of amyloid-beta protein, or intracellular neurotoxic aggregates consisting of hyperphosphorylated *tau (152)*. Both amyloid-beta and tau have been demonstrated as good *in vitro* substrates of TGase/TG2 (152). Phosphorylated tau accumulated in neurofibrillary tangles, as well as non-phosphorylated *tau* are substrates for TGase/TG2 (152). Also, a dysfunctional G-protein signaling caused by TGase/TG2's crosslinking of angiotensin II AT_2 receptor was shown to enhance the development of neurodegenerative symptoms in a transgenic animal model of *Alzheimers*' disease (153).

9.7. Wound healing and fibrosis

TG2 can be considered as a micromolecular suturing enzyme (biological glue) that enables tissues to resist proteolytic degradation and acquire enhanced mechanical strength (154). Both TG2 expression and activity were increased very early during wound healing demonstrated that the TG2 gene was induced and activated in cells that were migrating into the fibrin clot and/or remodeling the ECM. TG2 expression was found to associate with TGF-β, TNF-α, IL-6, and VEGF production in the wound. TG2 can also influence ECM biology by localizing cytokines and protease inhibitors (ECM stabilization phase) to the matrix (155-157). TG2 can crosslink elafin (a potent inhibitor for elastase) and alpha2-antiplasmin (a potent plasmin inhibitor) to ECM molecules (156, 158, 159). TG2 binds to beta-1 and beta-3 integrins (28, 160-163) and functions as a co-receptor to promote cell adhesion (28).

Under pathological conditions, TG2 exerts its effects at different phases of wound healing that lead to fibrosis. In the initial phase (trigger/inflammation), TG2 gene expression is induced by inflammatory cytokines (IL-1, IL-6, TNF-α and TGFβ (164-168) as damaged tissues respond attract inflammatory cells (168). TG2 can also serve as a receptor to recruit T cells into tissues which would further amplify injury responses (109). It is also possible that tissue injuries produce excessive TGFβ production resulting in TG2 production and extensive ECM crosslinking which leads to a microenviroment in tissues that promotes fibrosis.

9.8. Cytoskeleton's assembly and organization

Cytoskeletons are the backbone of cells that give rise to each cell's unique intracellular structure. Cytoskeletons also are crucial for cell division, motility and differentiation. Microtubules are formed by αβ-tubulin heterodimer building blocks that are post-translationally modified to give each cell's unique function. Tubulins undergo complicated post-translational modifications and TG2 is involved in these PTM (169). Tubulin is known to be a TGase/TG2 substrate but the physiological function of such modification remains unestablished (170). Polyamination of tubulin was recently found to play an important role in the stabilization of microtubule essential for unique neuronal function. (52). It is well-established that a fraction of neuronal tubulin is not soluble in cold and also resistant to calcium as well as drugs that depolymerize microtubules (171). Song *et al*. demonstrated that incorporation of spermine and spermidine into α-tubulin decreases the solubility of tubulins (52, 171). Polyamination by TG2 incorporates positive change into proteins and is known to make proteins more basic and insoluble. In the absence of polyamines, crosslinking of tubulins by guinea pig TG remains primarily in the soluble fraction (52). This is the first demonstration of modification of tubulins contribute the stability of microtubules (52). Polyamination by TGase/TG2 was postulated to contribute to the unique neurite formation in neuronal cells (52).

TGase/TG2-mediated PTM of actin has also been connected to insulin secretion (172), pollen tube growth (173), apoptosis (174), and neuronal cell form and function (175). Spermine was found to incorporate into actin derived from mouse tissues but the significance remains to be established (176).

TGase/TG2 mediated serotonylation of the RhoA and Rab4A GTPase is required for cytoskeletal rearrangement that leads to exocytosis of platelet α-granules, platelet activation and adhesion and platelet aggregation (38). The activation of RhoA by TG2 has also been demonstrated in RA-induced cells that involve the activation of Rock-2 kinase and formation of stress fibers and focal adhesion complexes (112, 177).

10. SUMMARY/PERSPECTIVE

Diseases associated with mitochondrial diseases normally associate with calcium dysregulation. TG2 represents one of calcium-activated enzyme with demonstrated pathophysiological important in mitochondrial disease. After synthesis, TG2 is localized in different cellular compartments including mitochondria and involved in mitophagy and mitochondrial function. TG2 participates in mitochondria's respiratory function, as TG -/- mice

had a serious defect in ATP production. Embryonic fibroblasts (MEFs) derived from TG2 -/- mice display mitophagy impairment associated with a metabolic shift towards aerobic glycolysis. High TG2 expressing breast cancer cells display a decrease in oxygen consumption rate (OCR) accompanied by an increase in extracellular acidification rate (ECAR) even under normoxic conditions. These data indicate a role of TG2 in metabolic reprogramming in normal and cancer cells. In a mitochondrial disease such in HD, activation of TGase/TG2 results in down regulation of the transcription of target genes, PPARγ coactivator 1α (PGC-1α) and downstream Cyt C, genes that are important for biogenesis of mitochondria. These data indicate a role of TG2 in mitochondrial biogenesis, degradation and metabolic reprogramming and warrant further investigation.

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