I. TRANSGLUTAMINASE: INTRODUCTION AND OVERVIEW

Transglutaminases (TGs; EC 2.3.2.13) are a family of structurally and functionally related proteins that catalyze the Ca²⁺-dependent posttranslational modification of proteins by introducing covalent bonds between free amine groups (e.g., protein- or peptide-bound lysine) and γ-carboxamide groups of peptide-bound glutamines (FIGURE 1). Researchers identified the first TG, now designated TG2, in 1959 from guinea pig liver extracts based on its ability to catalyze incorporation of low-molecular-weight primary amines into proteins (306). Since the discovery of TG2, additional proteins with this activity have been identified from unicellular organisms, invertebrates, fish, mammals, and plants (122). Nine TG genes are present in humans. Eight are catalytically active enzymes, and one is inactive (erythrocyte membrane protein band 4.2) (122). These proteins serve as scaffolds, maintain membrane integrity, regulate cell adhesion, and modulate signal transduction (TABLE 1) (308). Although the primary sequence of the TGs differ, with the exception of band 4.2, all share an identical amino acid sequence at the active site (FIGURE 2). In addition to the protein crosslinking and scaffolding functions, TGs catalyze posttranslational modification of proteins via deamination and amine incorporation (FIGURE 1). For example, TG2-dependent deamidation of gliadin A, a component of wheat and other cereals, is implicated in the pathogenesis of celiac disease (189). Similarly, deamidation of Gln63 in RhoA activates this signaling protein (108). Moreover, TG-catalyzed incorporation of amines into proteins can modify the function, stability, and immunogenicity of substrate proteins and contribute to autoimmune disease (220). Of the nine TGs identified in humans, TG2 is the most widely distributed and most extensively studied. In this review, we describe the role of TGs in general, and TG2 in particular, and also explore the consequences of aberrant TG expression and activation. TABLE 1 summarizes the general features of each member of the TG family.

A. Transglutaminase 1

Keratinocyte TG (TG1) is expressed in the stratified squamous epithelia of the skin and upper digestive tract and in the lower female genital tract. The TGM1 gene promoter contains three activator protein AP2-like response elements located ~0.5 kb from the transcription initiation site (238). Proteolytic cleavage, increased Ca²⁺ level, and interaction with tazarotene-induced gene 3 (TIG3) are known to activate TG1 catalytic activity (98, 156, 331, 332). Phorbol esters induce and retinoic acid reduces TG1 mRNA and protein expression (97). TG1 protein associates with the plasma membrane via fatty acyl linkage in the NH₂-terminal cysteine residue and is released by proteolysis as 10-, 33-, and 66-kDa fragments (183). Autosomal recessive lamellar ichthyosis results from mutation of the TG1-encod...
ing gene (46, 71, 140, 141). Common mutations include a C-to-T change in the binding site for the transcription factor Sp1 within the promoter region, a Gly143-to-Glu mutation in exon 3, and a Val382-to-Met mutation in exon 7. Lamellar ichthyosis is a rare keratinization disorder of the skin characterized by abnormal cornification of the epidermis. Individuals with ichthyosis exhibit drastically reduced TG1 activity and absence of detectable TG1 protein (46, 71, 140, 141).

**FIGURE 1.** Enzymatic reactions catalyzed by transglutaminases (TGs). Transamidation crosslinking reactions require the presence of Ca\(^{2+}\) to covalently link primary amines including polyamines, monoamines, and protein-bound amines (P2) to a glutamine residue of the acceptor protein (P1). These reactions form polyamines or monoamine crosslinks with proteins (1) or protein-protein crosslinks to form an \(\epsilon\)-(\(\gamma\)-glutamyl)lysine isopeptide bond (2). Under slightly acidic conditions, some TGs can utilize H\(_2\)O to catalyze deamidation of the P1 protein (3).

**B. Transglutaminase 2**

Tissue TG (TG2), also referred to as TGc or Gh, is widely distributed in tissues and cell types. TG2 is predominantly a cytosolic protein but is also present in the nucleus and on the plasma membrane (220). The TG2 gene promoter contains a retinoic acid response element (1.7 kb upstream of the initiation site), an interleukin (IL)-6 specific cis-regulatory element (4 kb upstream of the promoter), a transforming growth factor-\(\beta\)1 (TGF-\(\beta\)1) response element (868 bp upstream of the initiation site), and several binding sites for vitamin D receptors (320).

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**Table 1. Properties of transglutaminase proteins**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Protein</th>
<th>Chromosomal Location</th>
<th>Molecular Mass, kDa</th>
<th>Main Function</th>
<th>Tissue Distribution</th>
<th>Alternate Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGM1</td>
<td>TG1</td>
<td>14q11.2</td>
<td>90</td>
<td>Cell envelope formation during keratinocyte differentiation</td>
<td>Membrane-bound keratinocytes</td>
<td>T(_G)k, keratinocyte TG, particulate TG</td>
</tr>
<tr>
<td>TGM2</td>
<td>TG2</td>
<td>20q11-12</td>
<td>80</td>
<td>Apoptosis, cell adhesion, matrix stabilization, signal transduction</td>
<td>Many tissues: cytosolic, nuclear, membrane, and extracellular</td>
<td>T(_G)c, liver TG, endothelial TG, erythrocyte TG, Gh</td>
</tr>
<tr>
<td>TGM3</td>
<td>TG3</td>
<td>20q11-12</td>
<td>77</td>
<td>Cell envelope formation during keratinocyte differentiation</td>
<td>Hair follicle, epidermis, brain</td>
<td>T(_G)x, callus TG, hair follicle TG, bovine snout TG</td>
</tr>
<tr>
<td>TGM4</td>
<td>TG4</td>
<td>3q21-22</td>
<td>77</td>
<td>Reproduction, especially in rodents as a result of semen coagulation</td>
<td>Prostate</td>
<td>T(_G)x, androgen-regulated major secretory protein, vesiculase, dorsal prostate protein 1</td>
</tr>
<tr>
<td>TGM5</td>
<td>TG5</td>
<td>15q15.2</td>
<td>81</td>
<td>Cell envelope formation in keratinocytes</td>
<td>Foreskin keratinocytes, epithelial barrier lining, skeletal muscular striatum</td>
<td>T(_G)x</td>
</tr>
<tr>
<td>TGM6</td>
<td>TG6</td>
<td>20q11</td>
<td>78</td>
<td>Not known</td>
<td>Testis and lung</td>
<td>T(_G)z</td>
</tr>
<tr>
<td>TGM7</td>
<td>TG7</td>
<td>15q15.2</td>
<td>81</td>
<td>Not known</td>
<td>Ubiquitous but predominately in testis and lung</td>
<td>T(_G)z</td>
</tr>
<tr>
<td>F13A1</td>
<td>FXIIIa</td>
<td>6q24-25</td>
<td>83</td>
<td>Blood clotting, wound healing, bone synthesis</td>
<td>Platelets, placenta, synovial fluid, chondrocytes, astrocytes, macrophages, osteoclasts and osteoblasts</td>
<td>Fibrin-stabilizing factor, fibrinoligase, plasma TG, Laki-Lorand factor</td>
</tr>
<tr>
<td>EB42</td>
<td>Band4.2</td>
<td>15q15.2</td>
<td>72</td>
<td>Membrane integrity, cell attachment, signal transduction</td>
<td>Erythrocyte membranes, cone marrow, spleen</td>
<td>B4.2, ATP-binding erythrocyte membrane protein band 4.2</td>
</tr>
</tbody>
</table>
upstream), and two AP2-like response elements (634 and 183 bp upstream of the transcription initiation site). Retinoic acid, vitamin D, TGF-β1, IL-6, tumor necrosis factor (TNF), NF-κB, epidermal growth factor (EGF), phorbol ester, oxidative stress, and Hox-A7 induce TG2 expression. In addition to the transamidation reaction, TG2 displays GTPase, ATPase, protein kinase, and protein disulfide isomerase (PDI) activity. It interacts with phospholipase Cδ1, β-integrins, fibronectin, osteonectin, RhoA, multilin- eage kinases, retinoblastoma protein, PTEN, and IкBα. TG2 dysfunction contributes to celiac disease, neurodegenerative disorders, and cataract formation. TG2 knockout mice have no phenotype but display delayed wound healing and poor response to stress. Also, fibroblasts derived from TG2 mice display altered attachment and motility (351).

C. Transglutaminase 3

Transglutaminase 3 (TG3) or epidermal TG is present in hair follicles, epidermis, and brain. The TG3 gene (TGM3) promoter contains Sp1- and Ets-motifs (128 and 91 bp upstream of the initiation site, respectively), and expression of pro-transglutaminase 3 mRNA is increased by Ca2+. TG3 protein is encoded as two polypeptide chains derived from a single precursor protein by proteolysis. Like TG2, TG3 binds to and hydrolyzes GTP. It catalyzes the cross-linking of trichohyalin and keratin intermediate filaments to harden the inner root sheath of a hair follicle, which is critical for hair fiber morphogenesis (133–136, 162). It also participates in cell envelope formation during the latter stages of differentiation (162). TG3 knockout mice show impaired hair development and reduced skin barrier function (36, 162).

D. Transglutaminase 4

Transglutaminase 4 (TG4) or prostate TG is present in the prostate gland, prostatic fluids, and seminal plasma (91, 122, 160, 386). An Sp1-binding site, located −96 to −87 bp upstream of the transcription initiation site, is critical for transcriptional regulation of the TG4 gene expression, and androgen treatment increases TG4 mRNA level in the human prostate cancer cells. In rats, the enzyme participates in the formation of the copulatory plug in the female genital tract, and in masking the antigenicity of the male gamete. TG4 knockout mice exhibit reduced fertility due to defects in copulatory plug formation (84). The exact function of TG4 in humans is not known, but some recent reports suggest a link between increased expression of TG4 and promotion of an aggressive prostate cancer phenotype (160).

E. Transglutaminase 5

Transglutaminase 5 (TG5) is mainly expressed in foreskin keratinocytes, epithelial barrier lining, and skeletal muscle (53). The TG5 gene (TGM5) has a TATA-less promoter but contains putative binding sites for several transcription factors, including C-Myb, AP-1, NF-κB, and NF-1. GTP and ATP inhibit the protein crosslinking activity of TG5, whereas Ca2+ reverses this inhibition. In addition to full-length TG5 protein, three alternatively spliced isoforms of TG5 have been described: delta3 (deletion of exon 3), delta11 (deletion of exon 11), and delta3-delta11 (deletion of both exons). Full-length TG5 and the delta11 isoform are active, whereas delta3 and delta3-delta11 have low activity. TG5 crosslinks loricrin, involucrin, and SPR3 in epidermis (49) and contributes to hyperkeratosis in ichthyosis and psoriasis patients (48). TG5 inactivating mutations result in skin peeling syndrome (53). TG3 knockout mouse have not been generated.

F. Transglutaminase 6

Transglutaminase 6 (TG6) expression is localized in the human testes and lungs, and in the brain of mice. Human carcinoma cells with neuronal characteristics also express TG6. In addition to full-length protein, alternative splicing produces a short variant that lacks the second β-barrel domain (348). The catalytic function of TG6 is activated following proteolytic cleavage of the proenzyme; thus TG6 comprises two polypeptide chains that are cleaved from a single precursor. TG6 knockout mouse have not been generated.

G. Transglutaminase 7

Not much is known about TG7 gene regulation or function. Like TG6, expression is restricted to testes, lungs, and brain. One report suggested that TG7 transcript levels are increased in breast cancer cells of patients with poor prognoses (159). TG7 knockout mice are not available.

H. Factor XIIIa

Plasma TG (FXIIIa) is an important component of the blood coagulation cascade. It is found in platelets, plasma,
astocytes, macrophages, dermal dendritic cells, the placenta, chondrocytes, synovial fluid, the heart, the eyes, and in cells of osteoblast lineage. Expression of FXIIIA gene (F13A1) is regulated by a myeloid-enriched transcription factor (MZF1-like protein) and two ubiquitous transcription factors (NF1 and Sp1). Also, two myeloid-enriched factors (GATA-1 and Ets-1) induce F13A1 expression.

FXIIIA is the last zymogen activated in the blood coagulation cascade (220, 221) and is a heterotetramer composed of two A and two B subunits. The catalytic site of FXIII is localized in the A subunit, and the B subunit serves as a carrier protein. Upon activation by thrombin-dependent cleavage, the catalytic A subunit dissociates from the B subunit, yielding the active enzyme (FXIIIA). In the presence of Ca\(^{2+}\), the enzyme catalyzes crosslinking of fibrin molecules to stabilize fibrin clots.

FXIIIA also plays a role in inflammation and bone synthesis. Crosslinking of the AT1 receptor, catalyzed by FXIIIA, results in enhanced signaling and promotes monocyte adhesion in hypertensive patients, thereby accelerating atherogenesis. F13A1 deficiency is an autosomal recessive disorder characterized by a lifelong bleeding tendency and impaired wound healing. FXIIIA knockout mice have a clotting defect, increased incidence of miscarriage, decreased angiogenesis, and tissue remodeling defects (77, 193, 254, 355).

I. Erythrocyte membrane protein band 4.2 (Band 4.2)

Band 4.2 is a unique TG that lacks catalytic activity. A Cys-Ala substitution within the active site of band 4.2 is responsible for the lack of enzymatic activity (FIGURE 2). Band 4.2 is mainly present in erythrocytes, bone marrow, fetal liver, and spleen. Two isoforms of band 4.2 are produced by alternative splicing of the EPB4.2 gene; the shorter isoform is more abundant. Band 4.2 is a major component of the erythrocyte membrane cytoskeleton and plays an important role in maintenance of membrane integrity and regulation of cell stability. Band 4.2 binds to the cytoplasmic domain of the erythrocyte anion transporter (308). Band 4.2 protein expression is partially or completely absent in Japanese recessive spherocytic elliptocytosis patients. In these patients, the ankyrin protein is more loosely associated with the membrane skeleton than in normal individuals. Band 4.2 null mice show alterations in red blood cell function, including spherocytosis and altered ion transport (289).

Most tissues express multiple TG forms (www.ncbi.nlm.nih.gov/UniGene) and share common substrates (86). This may explain why TG family members can compensate for the loss of an individual enzyme. Perhaps the best-studied model for compensation is TGM2 gene knockout mouse.

Compensatory activation of the FXIIIa is observed in TG2+/− chondrocytes (266, 335), and TG1 and TG3 level and activity are increased in TG2+/− joint tissue (86). However, compensation is not observed in all tissues. For example, in skeletal muscle, loss of TG2 is not compensated (86).

II. REGULATION OF THE PROTEIN CROSSLINKING FUNCTION OF TG2

A. Regulation of TG2 Conformation

TG2, also known as tissue transglutaminase, cytosolic type II, or liver transglutaminase, is a unique member of the transglutaminase family of enzymes. In addition to Ca\(^{2+}\)-dependent posttranslational modification of proteins, it can also bind and hydrolyze GTP and acts as a G protein (220). Therefore, from catalytic activity point of view, TG2 can be referred to as bifunctional enzyme, owing to its ability to catalyze Ca\(^{2+}\)-dependent protein crosslinking activity and Ca\(^{2+}\)-independent GTP hydrolysis. Structurally TG2 is composed of four domains: an NH\(_2\)-terminal \(\beta\)-sandwich that contains integrin and fibronectin binding sites, a catalytic core domain which contains a catalytic triad (Cys277, His333, and Asp358) for acyl transfer reaction, and two COOH-terminal \(\beta\)-barrels. Although other members of TG family display a similar general structure, TG2 contains a unique guanine-binding site, located in the cleft between the catalytic core and the first \(\beta\)-barrel. This sequence is coded by exon 10 of the TGM2 gene. The spatial arrangement of the four domains in TG2 is altered by interaction with co-factors (FIGURE 3). For example, the GTP/GDP bound form displays considerable interaction between the catalytic domain and domains 3 and 4, which renders TG2 in a closed or compact conformation. This reduces accessibility and activity of the Ca\(^{2+}\)-dependent crosslinking site (217). In contrast, Ca\(^{2+}\) binding alters the conformation by moving domains 3 and 4 further apart, allowing TG2 to acquire an open/extended conformation and exposing the catalytic site. This open configuration is associated with the acyl transfer “crosslinking” reaction (293) (FIGURE 3). Crosslinking activity requires Cys277, which attacks γ-glutamyl residues on acyl donor substrates, on proteins and peptides, to drive formation of a thioester intermediate. The resulting acylated enzyme can then either react with an amine donor, typically an \(\epsilon\)-lysyl side chain of another protein/peptide, which associates with TG2 at a second substrate binding site. This results in isopeptide bond (crosslink) formation. Alternatively, in the absence of a suitable amine donor, the thioester is hydrolyzed to form glutamic acid, resulting in a net deamidation (FIGURE 1).

B. Allosteric Regulation of Transamidation Activity

TGs are present in intracellular and extracellular environments, and activity is tightly controlled under physiological...
conditions. For example, Ca\(^{2+}\), guanine nucleotides, and redox potential modulate TG2 crosslinking activity. Mutagenesis studies identify five potential Ca\(^{2+}\)-binding sites (188), and structure studies show that some of these sites are distorted when TG2 binds GTP/GDP (217). In essence, the protein can function as a G protein or as a transamidation enzyme. The transamination catalytic activity of TG2 is allosterically activated by Ca\(^{2+}\) and inhibited by GTP, GDP, and GMP. One molecule of TG2 binds up to six Ca\(^{2+}\) with an apparent overall dissociation constant of 90 \(\mu\)M (32). In contrast, GTP and GDP bind TG2 with a dissociation constant of 1.6 \(\mu\)M. GTP-bound TG2 cannot crosslink proteins, and crosslinking activity is only observed at high calcium Ca\(^{2+}\) concentrations. Because TG2 inside living cells is primarily GTP/GDP-bound, and calcium concentrations are low, it is believed that TG2 is predominantly present in a crosslinking-inactive form in cells. This may explain why overexpression of TG2 is not always associated with increased intracellular crosslinking activity. In a recent study, using TG2 that is covalently conjugated to enhanced yellow (YFP) and cyan fluoresce proteins (CFP) at NH\(_2\) and COOH terminus, respectively, Pavlyukov et al. (286) observed closed/inactive TG2 at a perinuclear location. In contrast, crosslinking-active TG2 was present at the cell membrane. Using the fluoresce resonance energy transfer (FRET)-based approach, these authors observed that TG2 changed from closed to open conformation in response to ionophore-induced calcium influx (286). In addition, Caron et al. (52) reported that an acrylamide-based TG2 inhibitor induces the open conformation, and a cinnamoyl triazole inhibitor stabilizes the closed conformation (52).

On balance, these observations support the contention that intracellular TG2 is predominantly present as a catalytically inactive form. Nevertheless, despite low intracellular calcium levels, multiple transamidation and crosslinking substrates of intracellular TG2 have been identified. This suggests that locally increased intracellular calcium and/or as yet uncharacterized interacting proteins may facilitate formation of open TG2. It should also be noted that some authors have suggested that relatively low calcium concentrations may be sufficient to activate TG2 crosslinking activity (173, 188). Finding additional TG2-binding proteins inside the cell (e.g., using yeast-2-hybrid or proteomics approach) and characterizing new TG2-interacting proteins under physiological conditions is expected to help address this issue.

A puzzling issue is why extracellular TG2 is inactive despite low GTP levels and high calcium levels (319). A possible explanation is the response of the enzyme to oxidative conditions in the extracellular environment. TG2 forms intramolecular disulfide bonds that are required for transamidation activity (37, 110), and a switch between the reduced (active) and oxidized (inactive) states of TG2 has been described (161, 327). This involves a triad of cysteine residues, including Cys370, Cys371, and Cys230, which have an unusually high redox potential (161). Mutation analysis and alkylation studies identified Cys230 as the key redox sensor. Under oxidizing conditions, an interstrand disulfide bond between Cys230 and Cys370 forms which facilitates formation of the more stable Cys370-Cys371 disulfide bond. These events inactivate the transamidation activity of TG2 (293, 327). In contrast, reduction of TG2 results in an open active conformation (327) (FIGURE 3). Thus the extracellular oxidative environment drives inactivation of its transamidase activity (73, 319). Another factor that con-

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**FIGURE 3.** Three TG2 conformations. Guanine nucleotide (GTP/GDP)-bound TG2 is compact (closed) and catalytically inactive. Catalytic activity refers to ability of the enzyme to perform the transamidation reaction. The structure of TG2 in its Ca\(^{2+}\)-bound form has not been resolved, but a putative Ca\(^{2+}\)-binding site homologous to FXIIIa is distorted by GTP/GDP binding to TG2. The binding of Ca\(^{2+}\) to the catalytic domain of TG2 alters the protein to move domains 3 and 4 away from the catalytic domain, thus making the active site accessible (open, catalytically active). Oxidation of the open/active protein results in loss of activity (open, catalytically inactive). The oxidized state can be prevented by treatment with thioredoxin. NH\(_2\)-terminal domain is blue. COOH-terminal domain is red.
tributes to inactivation of extracellular TG2 is nitric oxide (NO), which drives nitrosylation of several Cys residues in TG2 (202). In vivo, a gradual decrease in NO bioavailability during aging increases TG2 transamidation activity in blood vessels and increases their stiffness due to accumulation of crosslinks in the vascular extracellular matrix (ECM) (170, 303). Furthermore, protein kinase A phosphorylation of TG2(Ser216) stimulates TG2 kinase activity, while inhibiting transamidase function (245, 246). In contrast, thiol reductases (e.g., thioredoxin) activate extracellular TG2, thus antagonizing oxidative inactivation in the extracellular environment (161). Acting together, these factors modulate the redox, nitrosylation, and phosphorylation states of TG2 to control transamidase activity.

In summary, calcium, guanine nucleotides, and redox potential maintain mammalian TG2 activity in at least three distinct states depending on local conditions (FIGURE 3). The two most common TG2 states, the GTP/GDP-bound form and the Ca\(^{2+}\)-bound oxidized form, are catalytically inactive, whereas calcium binding activates the reduced form. Some thiol reductases, such as thioredoxin, are likely to control the redox state of extracellular TG2. In addition, the TG2 crosslinking activity is inhibited through nitrosylation and phosphorylation. The physiological implications of these allosteric regulatory and posttranslational modification mechanisms are described in subsequent sections. It is also important to note that protein-protein interactions regulate TG2 crosslinking activity. For example, in the ECM, TG2 interacts with a number of proteins, including fibronectin, osteonectin, and integrins (396), and interaction with some of these proteins alters enzymatic activity (359).

III. TRANSLUTAMINASE-REGULATED CELL SIGNALING

Although investigators originally discovered TG2 as a crosslinking enzyme, new functions have been identified. It is now appreciated that TG2 interacts with target proteins localized in the cytoplasm, membrane, ECM, nucleus, and mitochondria (151, 220, 278). This includes roles for TG2 in transamidation and protein-protein crosslinking, as a GTPase/ATPase, as a nonenzymatic adapter, as a scaffold protein, and as a regulator of signal transduction.

A. TG2 and FXIIIa Signaling: The Cell Surface and ECM

1. TG2 and FXIIIa crosslinking of ECM proteins

TG2 and FXIIIa are released into the extracellular environment via a poorly understood nonclassical secretion pathway (69, 398) where they covalently modify ECM proteins to form homo- and heteropolymers (3, 220) to enhance ECM stability (220). This crosslinking increases the rigidity of fibronectin (262) and collagen fibrils (326). The resulting increase in ECM stiffness enhances fibroblast and osteoblast adhesion (56, 112) and enhances cell survival, growth, migration, and differentiation by impacting integrin-related mechanosensing pathways (34). Endothelial cell adherence to the TG2-crosslinked fibrinogen αC increases integrin clustering and formation of focal adhesions, thereby elevating outside-in activation of focal adhesion kinase (FAK) and extracellular signal-regulated kinase (ERK) 1/2 activity (30). In addition, ECM protein crosslinking may expose cryptic integrin receptor-binding sites. For example, TG2-mediated polymerization of osteopontin creates a binding site for integrin α9β1 binding, leading to enhanced chemotactic migratory activity of neutrophils (264, 265). A similar mechanism influences vascular smooth muscle cell migration into FXIIIa-crosslinked fibrin gels (255), and impacts angiogenesis (138, 168).

2. Extracellular TG2 regulates the TGF-β signaling pathway

TG2-induced modification can modify growth factor activity in the extracellular environment (148, 220, 381). For example, TGF-β1 is a key regulator of ECM remodeling (387), and TGF-β1 activation involves integrins and proteases and is influenced by the oxidative environment and mechanical stress. TG2 covalently crosslinks latent TGF-β1-binding protein and thereby controls TGF-β1 maturation and activity (191, 362, 380). In addition, in fibroblasts, TG2 increases TGF-β mRNA and protein expression via a nuclear transcription factor (NF)-κB signaling mechanism (342). This results in a positive feedback loop in which TGF-β and TG2 display reciprocal activation of expression (29). In cancer cells, the TGF-β-induced increase in TG2 expression promotes epithelial-to-mesenchymal transition (EMT) (51, 196, 315).

3. Extracellular TG2 and FXIIIa enhance integrin-mediated signaling

Integrins are important transmembrane adhesion and signaling receptors that, although lacking intrinsic enzymatic activity, regulate a host of intracellular signaling pathways. Integrins are activated by binding to ECM (147). TG2 interacts with ECM to enhance cell adhesion and integrin-mediated signaling via direct interaction with β1, β3 and β5 integrin (FIGURE 4) (29, 396). TG2 also binds to the gelatin-binding region of fibronectin (297). The integrin-fibronectin binding is a weak interaction, while TG2 interacts strongly with both fibronectin and integrin, and thereby enhances integrin/fibronectin interaction. This facilitates cell attachment to the matrix and activates integrin signaling (29). TG2 controls integrin function in cancer cells (229, 309) and macrophages (29, 353). The interaction between integrin-bound TG2 and fibronectin is important in various disease conditions, including mesenchymal stem cell (MSC)
interaction with infarcted myocardium (325), cancer cell metastasis (309), and glial scarring (361).

The effect of TG2 on integrin function is evidenced by its impact on integrin clustering (155). The mechanism whereby TG2 promotes integrin clustering is not known; however, the ability of TG2 to oligomerize and interact with integrin-binding proteins, such as caveolin-1 and tetraspanins, may promote clustering. Moreover, localization of TG2 and β1-integrin in lipid rafts and caveolae (400) may enhance ECM interaction with these cholesterol-enriched membrane microdomains. Therefore, TG2 is likely to have a role in regulating membrane protein trafficking and compartmentalization during cell signaling.

TG2-induced integrin clustering potentiates integrin-dependent intracellular signaling (9, 155). This includes activation of FAK, Src, and p190RhoGAP and increased expression of active, GTP-bound RhoA and its downstream target, ROCK. The net impact of these events is increased focal adhesion and actin stress fiber formation leading to enhanced actomyosin contractility (FIGURE 4).

TG2 and integrins are also important in macrophages. TG2−/− macrophages are deficient in phagocytosis owing to altered accumulation of β3-integrin at the engulfing portals (353). Efficient signaling via β3-integrin, which is required for formation of the phagocytic cup and effective uptake of apoptotic cells, may require TG2 interaction with β3-integrin. TG2 activates downstream signaling targets of β3 integrin, including RhoG and Rac1, which are required for efficient phagocytosis. Furthermore, overexpression of β3-integrin in TG2−/− macrophages partially restores phagocytosis (354). Mechanistically, TG2 interacts with the protein milk fat globule EGF factor 8, which is involved in binding of β3-integrin to apoptotic cells, on the surface of macrophages. TG2-mediated stabilization of the β3-integrin/milk fat globule EGF factor 8 complex improves phagocytic uptake of apoptotic cells, likely owing to upregulation of β3 integrin-mediated activation of RhoG and Rac1 signaling.

FXIIIa, in contrast, is an integrin ligand and a covalent integrin modifier. Platelet integrin αIIbβ3 is the most common binding site for plasma FXIII (70) and serves as a transamidation substrate for platelet-derived FXIIIa (66). Of note, extracellular platelet FXIIIa suppresses Ca2+-dependent activation of αIIbβ3 integrin in cells that adhere to collagen in a transamidation-dependent manner, implying a role for FXIIIa in preventing excessive platelet accumulation on thrombogenic surfaces (194). In addition, plasma FXIII binds to integrin αvβ3 on endothelial cells and mediates platelet/endothelial cell interaction by bridging endothelial cell αvβ3 to platelet αIIbβ3 integrins (79). FXIIIa also stimulates endothelial cell, monocyte, and fibroblast proliferation and migration and inhibits apoptosis by interacting with αvβ3 integrin on the cell surface to trigger downstream signaling (76, 80). These effects of FXIIIa lead to increased vascularization and angiogenic actions of endothelial cells via activation of vascular endothelial growth factor receptor (VEGFR) 2, leading to increased expression of the Egr-1 and c-Jun transcription factors and downregulation of mRNA encoding the angiogenic ECM protein thrombospondin-1 (76, 78, 80).

The mechanism of FXIIIa-mediated activation of VEGFR2 in endothelial cells involves extracellular crosslinking of
this receptor to the β3 subunit of αvβ3, an integrin that facilitates angiogenesis (75). Extracellular FXIIIa promotes hypertrophic differentiation of chondrocytes by enhancing TG2 secretion (164). This effect does not require transamidating activity, but rather depends on FXIIIa interaction with αβ1 integrin via a novel integrin-binding site at the FXIIIa NH₂-terminus. Moreover, FXIIIa-dependent induction of type X collagen synthesis, a hallmark of chondrocyte differentiation, is mediated by α1β1-dependent activation of FAK and p38 MAPK signaling (165).

4. Modulation of syndecan-4 signaling by extracellular TG2

The heparan sulfate proteoglycan syndecan-4 localizes at points of cell-ECM contact, where it interacts via heparan sulfate with the Hep-2 region of fibronectin. It collaborates with integrins to enhance cell adhesion to fibronectin and facilitate adhesion-dependent RhoA-mediated development of focal adhesions, stress fibers, and actomyosin contractility (389). Syndecan-4 is an important TG2-binding partner (311, 344). A heparan sulfate binding site, Asn261LRRWKAsn265, that may mediate this interaction, is present in TG2 (366).

The high-affinity interaction of extracellular TG2 with syndecan-4 activates protein kinase C-alpha (PKCα), which, in turn, binds directly to the cytoplasmic tail of β1 integrin (FIGURE 4). This interaction controls integrin level and distribution on the cell surface as well as integrin stimulation of FAK and ERK1/2 (282, 311, 344, 379, 381). The ability of activated PKCα to maintain adhesion of fibroblasts and osteoblasts, via formation of ECM-based TG2-fibronectin complexes with cell surface syndecan-4, is mediated by syndecan-2 (379, 381). Syndecan-2 does not bind to TG2 but acts as a downstream signaling effector in modulating cytoskeletal organization via the ROCK pathway. These findings imply a major role for the TG2/fibronectin/syndecan-4 complex as an adhesive and signaling platform (363). The integrin- and syndecan-4-based adhesion systems are likely to physically interact with each other, as these two receptors bind to nonadjacent regions of fibronectin, and functionally collaborate by jointly regulating p190RhoGAP activity and localization during cell adhesion to the ECM (25, 343). Hence, this evidence indicates the existence of adhesion/signaling complexes composed of TG2, integrins, syndecan-4, and fibronectin. TG2 controls formation of these complexes owing to its high affinity for syndecan-4 and fibronectin.

Syndecan-4 interaction with integrin-bound TG2 at the cell surface and/or fibronectin-bound TG2 in the ECM may be required for response to tissue damage and ECM degradation. Thus increased TG2 expression during wound healing and tissue repair is likely to enhance cell adhesion and signaling to increase integrin-dependent adhesion and assembly of the fibronectin matrix (343, 367, 380). This may promote clustering of TG2 binding partners on the cell surface to enhance adhesion, prevent adhesion-mediated apoptosis (anoikis), and facilitate cell survival.

5. Regulation of growth factor receptor signaling by extracellular TG2 and FXIIIa

Physical association between integrins and receptor tyrosine kinases is required for the cell response to ECM and soluble growth factors (392). A novel example of a role for TG2 in this context is the interaction between integrin and platelet-derived growth factor receptor (PDGFR). TG2 interacts with PDGFR on the surface of fibroblasts and vascular smooth muscle cells, and enhances PDGFR interaction with integrins (397, 399) by bridging these receptors on the cell surface (FIGURE 4). The interaction with TG2 promotes PDGFR clustering, PDGF- and adhesion-induced PDGFR activation and downstream signaling, and PDGFR turnover. In particular, TG2 increases PDGF/PDGFR-mediated activation of Akt1 and Shp2 in fibroblasts and vascular smooth muscle cells (397). Cell surface TG2 is required for efficient PDGF-dependent fibroblast and vascular smooth muscle cell proliferation and migration. TG2 also enhances PDGF-induced vascular smooth muscle cell survival and suppresses differentiation. These studies revealed a novel function of cell surface TG2 in regulating PDGFR/integrin signaling and PDGFR-dependent cell responses, by coupling the adhesion-mediated and growth factor-dependent signaling pathways. These findings also suggest that TG2 activity may have a proinflammatory role in wound healing, tissue fibrosis, vascular restenosis, and tumor metastasis, diverse pathophysiological responses that often involve overactivation or dysregulation of the PDGF/PDGFR signaling axis (130).

The interaction of extracellular TG2 with growth factor receptors may be a general phenomenon since, as noted earlier, TG2 also binds to VEGFR on the surface of endothelial cells and modulates VEGF signaling (75). In this case, TG2 covalently crosslinks VEGFR to form high-molecular-weight complexes. In VEGF-treated cells, these complexes shuttle to the nucleus to enhance VEGF-induced ERK activation. Extracellular FXIIIa also regulates VEGF signaling by enhancing noncovalent interaction between VEGFR and αvβ3 integrin (75, 78). Future studies should identify the molecular motifs required for association of TG2 and FXIIIa with growth factor receptors, and address whether TG2 and FXIIIa interact with other structurally related receptor tyrosine kinases.

6. Extracellular TG2 as an activator of LRP5/6-mediated β-catenin signaling

Extracellular TG2 binds to the LRP5 and LRP6 (low-density lipoprotein receptor) transmembrane receptors on vascular smooth muscle cells (FIGURE 4) (102). Binding of TG2
to LRP5/6 triggers activation of the β-catenin pathway by driving nuclear translocation of β-catenin, inducing Tcf/Lef transcription factors and decreasing p21<sup>Cip1</sup> expression. TG2-mediated activation of β-catenin signaling promotes calcification of vascular smooth muscle cells (102). TG2 synergizes with LRP6 in the activation of β-catenin-dependent gene expression in COS-7 cells. Interfering with the LRP5/6 receptor function attenuates TG2-induced activation of β-catenin in these cells. Moreover, TG2 binds directly to the extracellular domain of LRP6 which acts as a substrate for TG2-mediated protein crosslinking (85). Future studies should assess the contribution of TG2-regulated LRP5/6 signaling to pathological conditions, such as cancer and calcification of blood vessels.

7. TG2 signaling via GPR56

GPR56 is an atypical G protein-coupled receptor (GPCR) that is reduced in level in metastatic melanoma cells, and interacts with cell surface-localized TG2 in tumor stroma cells (390). TG2 is proposed as a novel GPR56 ligand that cooperates in the growth-inhibitory and tumor-suppressive activity of GPR56; however, additional study will be necessary to understand the downstream signaling mechanisms involved in this activity.

B. Cytoplasmic TG2 and FXIIIa in Cell Signaling

1. TG-mediated monoaminylation of cytoplasmic proteins regulates signaling, the cytoskeleton, and vesicular trafficking

Monoamines, including serotonin, histamine, dopamine, and norepinephrine, are competitive inhibitors of TG cross-linking activity. However, these amines also can be utilized by TG to monoaminylate target proteins (FIGURE 5) (378). In this context, TG catalyzed serotonylation of the RhoA and Rab4A GTPases is required for cytoskeletal re-arrangement that leads to exocytosis of platelet α-granules, platelet activation, platelet adhesion, and platelet aggregation (377). Given that TG2 and FXIIIa are both abundant in platelets (220), knockout studies will be required to clarify which TG drives this reaction in vivo. In addition, serotonylation of Rab3A and Rab27A in pancreatic β cells is involved in the release of insulin (285). Although the TG that is involved is not known, the presence of missense mutations of the TGM2 gene in patients with early-onset type 2 diabetes mellitus is interesting (294). In one study, TG2 was identified to be the only TG significantly expressed in pancreatic β cells, and its deletion impaired glucose-stimulated insulin secretion (33). Thus, TG2 mutations (989T>G, 992T>A) that impair transamidation activity are linked with early onset of type 2 diabetes (294). However, although these mutations are not found in normal patients, heterozygous TGM2 mutations are not fully penetrant and do not appear to cause diabetes in these families. Iismaa et al. (150) evaluated the role of TG2 in diabetes and concluded that glucose homeostasis is TG2 independent and TG2 plays no role in pathophysiology of type 2 diabetes. Moreover, neither deletion nor activation of TG2 transaminidation activity in transgenic mouse models alters basal or insulin-challenged glucose homeostasis. This is clearly an area that will require future study.

In vascular smooth muscle cells, TG2-mediated serotonylation increases RhoA activity and degradation, which leads to increased Akt1 activity and inhibition of muscle contraction (124). TG2-mediated serotonylation of RhoA is also implicated in pulmonary arterial remodeling and hypertension (123). Moreover, TG2-mediated serotonylation of α-actin and other contractile apparatus proteins, in vascular smooth muscle cells, increases arterial isometric contraction (383). Similar TG2-mediated modification of smooth muscle proteins is observed during vasoconstriction (166). Moreover, TG2-dependent serotonylation activates Rac1 (another small GTPase) signaling in cortical neurons (74). In each of these examples, TG2-mediated incorporation of primary amines into cytoplasmic proteins influences activity, to alter the cytoskeleton and vesicular trafficking (FIG-
2. TG2-dependent regulation of NF-κB signaling

TG2 crosslinking regulates NF-κB signaling (181, 240). NF-κB belongs to a family of transcription factors that are important in inflammatory disease and cancer (178). Under normal cellular conditions, NF-κB is inactive in the cytoplasm because of its association with IκBα. Exposure to stress stimuli activates pathways that ubiquitinate IκBα via a mechanism that involves IκB kinase (IKK)-dependent IκBα phosphorylation (23). Proteasome-mediated degradation of IκBα releases NF-κB, which translocates to the nucleus to activate gene expression (121) (FIGURE 6A). IKK-independent NF-κB activation, via a mechanism that involves TG2 crosslinking of IκBα, has recently been described (FIGURE 6B) (205, 230). TG2-mediated polymerization of IκBα results in IκBα proteasomal degradation, leading to the NF-κB activation (205). TG2 also interacts directly with IκBα to cause IκBα degradation via a nonproteasomal mechanism (198) (FIGURE 6B). These novel TG2-mediated, IKK-independent mechanisms of NF-κB activation are important and suggest that targeting these events may block inflammation (181, 370).

3. TG2-crosslinking of PPAR-γ links oxidative stress and inflammation

Cystic fibrosis is caused by mutation of the cystic fibrosis transmembrane conductance regulator (CFTR) leading to chronic airway inflammation. Interestingly, human bronchial epithelial cells that express functionally deficient CFTR express high levels of TG2, leading to increased crosslinking and sequestration of anti-inflammatory PPAR-γ. This suggests a role for TG2 in mediating the inflammatory response in cystic fibrosis patients (227). TG2 crosslinking promotes accumulation of polymerized ubiquitinated PPAR-γ in perinuclear aggresomes and, as a result, PPAR-γ interaction with the N-CoR-histone deacetyl-

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**FIGURE 6.** TG2 expression results in constitutive activation of NF-κB via noncanonical pathway. Acute inflammation is a tightly regulated physiological process in which NF-κB is transiently activated as a result of IKK-complex mediated phosphorylation and degradation of the inhibitory protein IκBα. As IκBα is one of the downstream targets of NF-κB, its expression results in feedback inhibition of NF-κB, which limits the inflammatory response (4). In contrast, chronic inflammation is associated with constitutive activation of NF-κB owing to aberrant expression of TG2 (B). TG2 binds to IκBα resulting in its rapid degradation via a nonproteasomal pathway. Alternatively, TG2-mediated covalent crosslinking of IκBα may promote proteasomal degradation of IκBα polymers (broken arrows). TG2-activated NF-κB regulates the expression of multiple target genes that play roles in cell survival, invasion, and drug resistance. One of the TG2/NF-κB target genes is HIF-1α, a transcription factor known to promote an aggressive phenotype in cancer cells.
lase 3 complex is reduced, thereby facilitating inflammatory response gene expression (283). Moreover, inhibition of TG2-mediated crosslinking restores normal PPAR-γ level and reduces inflammation in both cultured CFTR-defective cells and cystic fibrosis tissue (227).

Oxidative stress in CFTR-defective cells also increases SUMO ligase activity. SUMO ligase inhibits activated STAT-γ leading to reduced TG2 SUMOylation which reduces TG2 turnover and increases TG2 level and activity (224). This evidence is consistent with the finding of elevated reactive oxygen species and increased TG2 SUMOylation in lung tissue in mutant ΔPhe508-CFTR mice, a model of cystic fibrosis, and suggests that control of TG2 turnover serves as a central link between oxidative signaling and inflammation in cystic fibrosis (223, 224). These findings established cytoplasmic TG2 as a novel mediator that connects oxidative stress and inflammation.

4. TG2 crosslinking of beclin-1 and inhibition of autophagy

In addition to an impact on protein aggregation, stress-induced accumulation of cytoplasmic TG2 and activation of TG2 crosslinking inhibits autophagy. Specifically, PKCδ-mediated induction of TG2 expression in pancreatic carcinoma cells inhibits autophagy by crosslinking beclin-1 to inhibit its function (7, 277). This mechanism also operates in CFTR-deficient lung cells where oxidative stress-related TG2-induced crosslinking of beclin-1 leads to sequestration of beclin-1, and beclin-1 interacting proteins, in the aggresomes (223). These findings suggest a central role for TG2 in beclin-1 depletion, beclin-1 sequestration in aggresomes, and inhibition of autophagy, in patients with cystic fibrosis.

5. Cytoplasmic TG2 and EGF/EGFR signaling in epithelial cancer cells

EGFR activity, which is frequently increased in human malignant cells, increases TG2 expression in cervical, breast, and lung epithelial cancer cells (15, 214). Moreover, induction of TG2 expression and TG2-dependent transamidation are essential for EGF-mediated migration, invasion (15), and anchorage-independent cancer cell growth (213). The EGF-induced response is mediated by Ras- and Cdc42-induced activation of PI3K and NF-κB and requires TG2-mediated upregulation of Src expression (15). TG2-induced Src expression is associated with transamidation-dependent formation of cytoplasmic ternary complexes of Src, TG2, and keratin 19 (213). EGF signaling, via Ras and JNK, causes TG2 activity to accumulate at the leading edge of cells. Accumulation of cytoplasmic TG2 at this location is necessary for cell migration and requires interaction of TG2 with heat shock protein 70 (Hsp70) (38). Similarly, EGF-induced upregulation of TG2 expression in TNF-related apoptosis-inducing ligand (TRAIL)-resistant lung cancer cells elevates MMP-9 expression, secretion, and activity, and this enhances the migration and invasiveness of these cells (214). The mechanism of TG2 action in this context remains to be defined; however, JNK/ERK signaling pathways are implicated in this process (214). Thus cytoplasmic TG2 is a novel mediator of EGF/EGFR-induced signaling and oncogenesis in epithelial cancer cells that involves TG2 transamidation-dependent and -independent actions.

6. Regulation of angiotensin signaling by FXIIIa-induced dimerization of AT1 receptors

GPCRs constitute a large family of cell surface receptors. GPCR homodimers and heterodimers influence many receptor-related functions, including ligand binding, membrane localization, signaling, and desensitization (116). However, until recently, little was known about the pathophysiological importance of GPCR dimerization in vivo. An insightful study revealed a novel mechanism of FXIIIa-mediated dimerization of the angiotensin II AT1 receptor in monocytes (1). Crosslinking of AT1 dimers, via glutamine residues, in the tail domain enhances receptor signaling. Moreover, FXIIIa-deficient individuals lack crosslinked AT1 dimers, whereas patients with the common atherogenic risk factor hypertension have elevated levels of these dimers. The presence of these dimers correlates with enhanced adhesion of angiotensin II-stimulated monocytes to endothelial cells (1). Importantly, in monocytes, these AT1 dimers promote atherogenesis, and inhibition of FXIIIa crosslinking activity reduces AT1 dimer formation and reduces disease severity in atherosclerosis in mice. Thus FXIIIa, via an impact on AT1 receptors, appears to have a role in maintaining atherogenesis.

7. FXIIIa-mediated crosslinking of Glu-tubulin alters microtubule dynamics and controls osteoblast matrix deposition

The transamidating activity of TG2 and FXIIIa, accompanied by collagen type I and fibronectin deposition into the ECM, is associated with osteoblast differentiation. However, the molecular mechanisms linking these events remain largely unknown (266). A recent study showed that inhibition of FXIIIa-mediated transamidation in osteoblasts resulted in microtubule destabilization as evidenced by reduced Glu-tubulin levels and blocked formation of Glu-tubulin oligomers (11). In turn, blockage of this activity inhibited vesicle-based secretion and deposition of collagen type I and fibronectin. Thus this study provides potential mechanistic clues regarding the role of transamidation and protein crosslinking by FXIIIa in the regulation of ECM protein secretion and deposition that leads to osteoblast differentiation.
8. Cytoplasmic TG2 as an atypical GTPase and mediator of GPCR-induced signaling

Although the discovery that TG2 can bind to and hydrolyze GTP occurred in 1987 (2), researchers did not establish a link between this activity and GPCR function until 1994 when a GTP-binding protein, termed Gho, was isolated with the α1B adrenergic receptor. The study showed that Gho was TG2 (Figure 7) (259). Other researchers observed a similar role for TG2 relative to the α1D adrenergic, thromboxane A2, oxytocin, and follicle-stimulating hormone receptors. In response to exposure to agonists of these receptors, PLCβ1 activation leads to increased inositol 1,4,5-trisphosphate level (19, 20, 104, 153, 220, 241, 279, 373).

The GTPase activity and associated signaling capacity of TG2 is independent of its transamidating activity (58). Moreover, given the high intracellular GTP levels observed under normal physiological conditions, the activity of TG2/Gho as a GPCR-linked GTPase is physiologically relevant. TG2 binds to and hydrolyzes GTP with an affinity and catalytic rate similar to that observed with the canonical α subunits of heterotrimeric and monomeric G proteins despite the absence of the four consensus GTP-binding motifs common to the classical G proteins. Mutating Arg580 in the large-conductance Ca²⁺-activated K⁺ channels in vas-

![FIGURE 7. TG2 GTPase activity and TG2/Gho signaling. The GDP-TG2/Gho/CRT/Ghβ complex is inactive. CRT is calreticulin. 1: Agonist stimulation of transmembrane G protein-coupled receptors (GPCR) induces exchange of GDP with GTP and dissociation of GTP-bound TG2/Gho from CRT/Ghβ. 2: GTP-bound TG2/Gho activates PLCδ1. 3/4: Signal termination occurs with GTP hydrolysis and reassociation of GDP-bound TG2/Gho with free CRT/Ghβ. 5: PLCδ1 promotes coupling efficiency by stabilizing GTP-TG2/Gho. 6: PLCδ1 catalyzes hydrolysis of phosphatidylinositol 4,5-bisphosphate to diacylglycerol and inositol 1,4,5-trisphosphate, causing an increase in intracellular Ca²⁺ level. 7: Switching off GTPase activity of TG2/Gho is triggered by elevated intracellular Ca²⁺.

TG2 functions similarly to that of other heterotrimeric G proteins (Figure 7) (220, 241). Agonist binding induces exchange of GDP with GTP and dissociation of TG2/GTP from Ghβ. Deactivation occurs when TG2 hydrolyzes GTP to GDP and reassociates with free Ghβ. Two regions in TG2, R564-D581 and Q633-E646, are involved in TG2 interaction with α1 adrenergic receptors and activation of the GTPase function (103).

The role and specificity of TG2 in GPCR signaling is determined not only by the range of receptors it interacts with but also by the downstream effectors. PLCδ1 is a key downstream target of TG2/α1 adrenergic receptor coupling (21, 81, 104). The Val665-Lys672 region in the COOH terminus of TG2 is involved in binding and activation of PLCδ1 (146), which hydrolyzes phosphoinositide and increases intracellular free calcium level (104, 175). PLCδ1 acts as both a guanine nucleotide exchange factor and a GTP hydrolysis-inhibitory factor for TG2 (21). TG2 also regulates other signaling pathways via its GTPase activity. It participates in ERK1/2 activation in cardiomyocytes (206). In fibroblasts and endothelial cells, overexpression of TG2, or transamidation-inactive TG2, inhibits adenylyl cyclase activity, whereas knockdown of TG2 reverses this effect (119). TG2 also increases adenylyl cyclase activity in neuroblastoma cells, but this effect requires its transamidating activity (356), implying that TG2 can regulate signaling pathways differentially dependent upon cell type. Also, TG2 activates the large-conductance Ca²⁺-activated K⁺ channels in vas-

PLC
cular smooth muscle cells (207), and GTP-bound TG2 binds to the cytoplasmic tail of α5 integrin to inhibit vascular smooth muscle cell migration (177). In contrast, TG2 promotes fibroblast migration via its GTP-binding activity (330). Finally, TG2 GTPase activity regulates cell-cycle progression in fibrosarcoma cells (242) and mediates α1 adrenergic receptor-induced proliferation of hepatocytes (388) and visceral smooth muscle cells (92).

Despite significant progress in understanding of the GTPase function of TG2, the pathophysiological role of the associated intracellular signaling remains poorly understood. For instance, cardiac-specific overexpression of TG2 fails to alter PLCδ1 activity, suggesting that TG2 acts as a TG rather than a GTPase in this context (323). Nonetheless, TG2 GTPase activity is markedly reduced in ischemic heart, suggesting that loss of this activity may be an important factor in cardiac failure (146). TG2 GTPase activity may also be involved in liver regeneration owing to its involvement in α1 adrenergic receptor signaling (304, 388). Overall, TG2 GTPase signaling is prosurvival and cytoprotective, as mutants of TG2 defective in GTP binding appeared to induce apoptosis in NIH3T3 and HeLa cells independent of their transamidating activity (82). Moreover, the GTPase function (FIGURE 3), and intracellular localization of TG2, are important in protecting cells from death caused by oxygen and glucose deprivation (67, 126).

C. Signaling Function of TG2 in the Nucleus

1. Nuclear TG2 transamidation and regulation of gene expression

TG2 nuclear localization was initially reported in hepatocytes (128). Nuclear TG2 comprises ~5% of the total TG2 cellular pool (278). TG2 displays crosslinking and GTPase activity in this cellular compartment (321) and associates with chromatin (209). The crosslinking activity of nuclear TG2 has a role in histone and transcription factor transamidation which alters gene expression (236, 340). Perhaps the best example of transamidation regulation of gene expression is the TG2 impact on Sp1 function (FIGURE 8B) (317, 339, 340). Sp1 is involved in alcohol-induced apoptosis, a process in which TG2 crosslinks Sp1 resulting in Sp1 inactivation (339). This leads to decreased expression of growth factor receptors, such as c-Met, which results in cell death. TG2 regulation of Sp1 function is also observed in free fatty acid-treated hepatocytes and in patients with nonalcoholic steatohepatitis (340).

2. TG2 as a transcriptional coregulator

Recent reports indicate that nuclear TG2 functions nonenzymatically as a transcriptional coactivator (5, 106). TG2-dependent reduction in MMP-9 gene transcription in cardiomyoblasts is mediated by direct noncovalent binding of TG2 to c-Jun, thereby inhibiting c-Jun/c-Fos dimerization and blocking interaction with the AP-1 site in the MMP-9 gene promoter (FIGURE 8C) (5). A similar mechanism operates in cortical neurons where nuclear TG2 interaction with HIF-1β prevents dimerization with HIF-1α (FIGURE 8D) (106). This interaction attenuates expression of Bnip3 and other genes containing the hypoxia-response element (HRE) to reduce neuron death in patients with ischemia and reduce stroke. In addition, the protein kinase activity of nuclear TG2 may be involved in the phosphorylation of histones H1 and H3 (248), p53 (247), and retinoblastoma protein (245) (FIGURE 8A).

D. Signaling by Mitochondrial TG2

Although TG2 does not have a classical NH2-terminal mitochondrial targeting signal, the protein does associate with mitochondria in various cell types (291, 299). In the majority of cells, mitochondrial TG2 localizes at the outer mitochondrial membrane and the inner membrane space; however, in 10% of cells, TG2 is present at the inner mitochondrial membrane and mitochondrial matrix (278, 299).

1. TG2 crosslinking of mitochondrial proteins is involved in the mitochondrial-driven apoptosis

The TG2 sequence includes LKNAGRDC211, which is 70% homologous to the BH3 domain of Bcl-2 family proteins, suggesting that TG2 is a novel apoptotic BH3 protein (299). Mutation of the highly conserved Leu204 residue in this motif attenuates TG2-mediated staurosporin-induced neuroblastoma cell death, confirming earlier findings that TG2-induced hyperpolarization of the mitochondrial membrane sensitizes cells to the intrinsic pathway of programmed cell death (291, 299). Also, the TG2 BH3 peptide interacts with proapoptotic Bax but not with anti-apoptotic Bcl-2, and TG2-Bax interaction increases during cell death. In contrast, TG2 inhibits calcium-induced apoptosis in HEK293 cells by covalently crosslinking Bax and down-regulating Bax expression (61). Thus the proapoptotic or antiapoptotic function of mitochondrial TG2 may depend on the cell type and cell death inducer.

In addition, TG2-mediated transamidation of proteins in mitochondria has been reported (278, 305). Prohibitin is a membrane-bound chaperone essential for correct folding of the Hsp70 and Hsp90 respiratory chain components. The organizing protein Hsp60 cooperates with prohibitin and 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. In apoptotic neural cells, these proteins are transamidated and crosslinked by TG2 (26, 275). 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, is also crosslinked by TG2 (228). In general, TG2-mediated covalent modification of mitochondrial proteins does not occur in normal tissue; however, modification is likely in patients with "mitochondrial diseases," including cardiovascular ischemia/reperfusion injury and neurodegenerative disorders such as Huntington disease. Accordingly, TG2-catalyzed crosslinking of mitochondrial matrix α-ketoglutarate dehydrogenase (68) and aconitase (186) is observed with decline in energy metabolism. Moreover, high-molecular-weight aggregates of these enzymes are observed in Huntington disease patients having elevated TG2 crosslinking activity. Additional studies are needed to establish the precise role of TG2-mediated crosslinking in the mitochondrial apoptosis pathway.

2. TG2 PDI enzymatic activity and the mitochondria

TG2 also acts as a PDI to regulate mitochondrial function (228, 231, 305). Deletion of TG2 leads to defective disulfide bond formation in NADH-ubiquinone oxidoreductase (complex I), succinate-ubiquinone oxidoreductase (complex II), cytochrome c oxidase (complex IV), and ATP synthase (complex V). TG2 PDI activity may also control the respiratory chain by modulating protein complex formation (231). Another target of TG2 PDI activity is ANT1...
(228). ANT1 oligomerization is essential for activity, and TG2−/− mice have increased thiol-dependent ANT1 oligomer formation and elevated ANT1 ADP/ATP exchange activity in heart mitochondria. Thus the PDI activity of TG2 reduces the level of oligomerized ANT1 and inhibits transporter activity by sequestering ANT1 monomers and preventing oligomer formation by direct binding to ANT1. In addition, the presence of TG2 is required for Bax/ANT1 colocalization and interaction in mitochondria (228). Taken together, these findings reveal an important role for TG2 PDI enzymatic activity in vivo and indicate the existence of a novel pathway that links this activity with regulation of mitochondrial physiology.

Future studies to understand TG2 trafficking and shuttling among various cellular compartments is important. How do cells regulate these trafficking events? What are the TG2 molecular signature motifs (e.g., “barcodes”) for its targeted delivery to different organelles? What are the compartment-specific binding partners of TG2? Which signaling pathways regulate distribution? Defining these issues should help to determine the compartment-specific functions of TG2. For example, the vexing lack of understanding of how intracellular TG2 moves to the cell exterior (398) is an important example of a trafficking mechanism that needs to be clarified.

IV. TGS IN CELL DIFFERENTIATION

TG activity is fourfold higher in blastocysts than in two-cell embryos (225), suggesting that transglutaminases have an important role in development. Little is known about which TG isoforms are responsible for this activity. However, more is known about the role of transglutaminases in specific tissues which will be discussed in this section.

A. Epidermis

The epidermis is a stratified epithelium formed by specialized cells called keratinocytes (94, 95). It consists of the basal, spinous, granular, and cornified layers. The proliferating cells are in the basal layer, and the spinous and granular and cornified layers display progressive differentiation (95). The cornified layer consists of completely differentiated cells that form the body surface. At the cellular level, the final stage of keratinocyte differentiation begins in the epidermal spinous and granular layers with accumulation of cornified envelope precursors on the inner surface of the plasma membrane (55, 95, 96, 99). These proteins are crosslinked by TGs to form the rigid cornified cell envelope that gives the differentiated keratinocyte its protective properties (46, 329). TG crosslinks cornified envelope precursors including involucrin, loricrin, filaggrin, and small proline-rich proteins (50, 95, 97, 132).

At least four TGs are expressed in human epidermis: TG1, TG2, TG3, and TG5. TG2 is detected in basal keratinocytes, although the precise role of TG2 in skin cells is not known (48, 184). TG1 and TG3 and TG5 are produced in the differentiated cells of the spinous and granular layers (48, 184) where they act on substrates to assemble the cornified envelope (50, 95, 391). In addition, TG1 mediates covalent crosslinking of ceramides to involucrin, which contributes to maintenance of the epidermal permeability barrier (263).

TG1 and TG5 mutations produce defects in the cornification process. Mutation of TG1 in either the catalytic cysteine, or surrounding region, is associated with lamellar ichthyosis (140, 281, 302, 346). A similar phenotype is observed in mice lacking the TG1 gene (234). These mice have defects in epidermal barrier formation and die shortly after birth. Interestingly, other TGs cannot functionally replace TG1 action despite their ability to crosslink loricrin, involucrin, and other precursors (47). Similar to the critical role of TG1 activity in cornification, a loss-of-function mutation of TG5 is associated with acral peeling skin syndrome (53). In contrast, a role for TG2 in keratinocyte differentiation has yet to be demonstrated, and TG2-knockout mice do not have any obvious skin defects (261). Likewise, the role of TG3 in keratinocyte differentiation remains to be elucidated.

B. Nervous System

1. Neuronal differentiation

Dendritic extension and axonal branching are key features of neurogenesis and often used as markers of neuronal differentiation, and catalytically active TG2 may modulate the rate and extent of neurite outgrowth. For example, an increase in total TG activity is associated with neurite outgrowth in murine neuroblastoma cells (226), and forced expression of TG2 in SH-SY5Y neuroblastoma cells causes spontaneous neurite outgrowth and neuronal marker expression following retinoic acid treatment (322). In contrast, elevated expression of transamidating-inactive (C277S) TG2 represses neuroblastoma cell differentiation (341). These findings suggest a requirement for the TG2 transamidating function in this process.

The specific molecular mechanisms underlying induction of neuronal differentiation by the transamidating activity of TG2 are emerging. TG2 protein is prominently localized at the tips of outgrowing neuritis, suggesting that it has a role in stabilizing extended structural projections (357). In addition, a role for TG2-dependent activation of c-Jun NH2-terminal kinase (JNK) signaling in neurite outgrowth has been proposed (322), as has activation of adenyl cyclase by the transamidating active form of TG2, leading to protein kinase A-mediated CREB activation (356). This mechanism seems to be specific to neuronal differentiation, because TG2 inhibits adenyl cyclase activity and decreases...
cAMP levels in Balb-c 3T3 human fibroblasts and bovine aorta endothelial cells (119), and in MSCs undergoing chondrogenic differentiation (270).

In contrast to the proposed role of the transamidating activity of TG2 in neuronal differentiation, the role of this enzyme in neurodegeneration is not clear. Neuronal TG2 attenuates cell death in ischemic conditions (106, 107), but facilitates cell death in excitoxic conditions (358). Thus TG2 accumulation in brains of Parkinson, Huntington, and Alzheimer’s patients may be a compensatory, protective response (158, 163, 185, 211).

2. Macroglia

Glia cells maintain homeostasis, form myelin, and support and protect neurons. Similar to neurons, glial cells originate in the neural tube and neural crest of the developing embryo. The most common type of macroglial cell is the astrocyte which has numerous projections that help anchor neurons to the blood supply. They also secrete regulatory proteins that promote the myelinating activity of oligodendrocytes, another abundant type of microglial cell. Oligodendrocytes form a specialized myelin membrane, which coats and protects axons in the central nervous system. Genetic ablation of TG2 results in delayed remyelination in injured animals, suggesting a role for TG2 in glial differentiation. In addition, in vitro studies demonstrated that TG2 regulates astrocyte migration, which is required for proper remyelination in vivo and for differentiation of myelin-producing oligodendrocytes from precursor oligodendrocytes (361). Moreover, both of these processes are attenuated by the TG2-specific inhibitor KCC009 [(S)-3-(4-hydroxyphenyl)-2-N′-(phenylmethoxy carbonyl)aminopropionic acid N′-(3′-bromo-4′,5′-dihydro-5′-isoxoyl)methylamide] (361). The precise mechanism of TG2 action remains to be determined, but reported data implicate a role for RhoA in TG2-dependent differentiation of oligodendrocytes (360).

C. Immune System

Accumulating evidence indicates an important role for TG2 in cell-mediated immunity and suggests that TG2 contributes to the immune response via regulation of differentiation in phagocytes, monocytes, neutrophils, and T cells. During monocyte differentiation, expression of FXIIIa and TG2 is induced and may contribute to neutrophilic and monocytic differentiation (8, 208, 251, 252). Genetic and pharmacology studies identify TG2 as a positive regulator of neutrophilic differentiation. For example, genetic ablation of TG2 in murine neutrophils results in diminished superoxide anion production and impaired extravasation, indicative of delayed differentiation (22). Moreover, TG2 silencing in the human promyelocytic leukemia cell line NB-4 leads to a significant delay in the neutrophil differentiation process and downregulation of expression of genes related to the innate immune system (72). Similar to its effects on neutrophilic differentiation, TG2 activity plays an important role in monocytic differentiation to macrophages and dendritic cells, the antigen-presenting cells that act as messengers between innate and adaptive immunity. TG2 regulates cell adhesion and migration in differentiating macrophages (8, 251), whereas in dendritic cells, TG2 mediates maturation of antigen-presenting cells in response to bacterial lipopolysaccharide (LPS) (233). Moreover, inhibition of TG2 with KCC009 (62) reduces cytokine production and dendritic cell differentiation in vitro (233). Altered resistance to LPS-induced septic shock in TG2-null mice further confirms that TG2 has a significant role in dendritic cell maturation (233). In addition, FXIIIa may regulate terminal maturation in both macrophages and dendritic cells, and FXIIIa-deficient human monocytes demonstrate reduced phagocytosis (307). In contrast, FXIIIa overexpression results in enhanced dendritic cell motility (157).

D. Connective Tissues

Connective tissues, including cartilage, bone, adipose tissue, ligaments, and tendons, differentiate from MSCs (17, 18, 325). TG2, but not FXIIIa, is expressed in MSCs (325); however, FXIIIa expression is induced in cells of several lineages during differentiation. Accumulating evidence suggests a role for TGs in regulation of MSC differentiation. TG2 can crosslink and stabilize cytoskeletal proteins including actin, tubulin, vimentin, and myosin, suggesting a role in establishing cellular phenotype (65, 87, 101). In addition, extracellular TG2 stabilizes the cytoskeleton by promoting integrin clustering and regulating FAK, RhoA GTPase, ROCK, and mitogen-activated protein kinase (MAPK) activity (155). TG2-mediated crosslinking increases the stiffness of the ECM (262, 314, 326), and this is sensed by integrin receptors (43, 180) and converted into signaling output (316). MSC differentiation is controlled by matrix stiffness (100, 137, 260), and TG2-modified collagen scaffolds promote chondrogenic differentiation of human MSCs (314). Extracellular matrices with “high stiffness” favor osteoblast differentiation over neural, muscle, and chondrogenic differentiation (100, 260). Thus MSC-associated TG activity, both intracellular and extracellular, regulates MSC differentiation.

1. Cartilage and chondrocytes

Endochondral bone formation is a key event in skeletal development. This is a process in which cartilage is first generated and then replaced by bone (219). The first step in endochondral bone formation is chondrocyte differentiation, which begins with MSC condensation and progresses through a series of maturation stages including resting, proliferative, prehypertrophic, and hypertrophic stages (219). Terminal differentiation of chondrocytes is characterized by deposition of mineralized ECM that is associated with
cell death and replacement of cartilage with bone. TG2 regulates the transition to the prehypertrophic stage, which is characterized by cessation of ECM deposition and reduced cell proliferation (270). Expression of FXIIIa and TG2 is markedly increased during this transition (267, 270), and both enzymes are found in the intracellular and extracellular compartments during terminal differentiation (4, 268, 349). Premature expression of TG2 in cultured MSCs accelerates prehypertrophic differentiation and inhibits deposition of the cartilaginous matrix, whereas treatment with the TG2 inhibitor ERW-1065 (382) enhances the matrix synthesis (270). In vivo, increased TG2 expression delays endochondral ossification in chicken embryos by blocking chondrocyte maturation during prehypertrophic differentiation. TG2-mediated inhibition of protein kinase A activity is implicated as a major mechanism underlying this regulation.

In contrast to endochondral cartilage, which is replaced by bone, articular cartilage remains cartilaginous throughout life. Articular chondrocytes are maintained in the resting stage and do not undergo proliferation or terminal differentiation (90). Both TG2 and FXIIIa are expressed by healthy articular chondrocytes (301, 349) and may block chondrocyte differentiation. In addition, a role for TG2 and FXIIIa in cell response to mechanical stress and abrasive force can be postulated based on the pattern of FXIIIa expression and localization of TG activity to condylar regions (269).

Diseases and conditions such as osteoarthritis and aging are associated with increased expression of both TG2 and FXIIIa. In vitro studies have shown that extracellular TG2 enhances hypertrophic differentiation and induces matrix mineralization in cultured articular chondrocytes via α5β1-integrin-mediated activation of Rac1 and p38 in a fibronectin-independent manner (165, 335). Interestingly, these effects require the GTP-bound form of TG2. Whether this mechanism is active in vivo where extracellular TG2 may not be GTP-bound, remains to be determined. TG2 mobilization to the cell surface is regulated by FXIIIa interaction with α1β1-integrin. Thus atypical maturation of articular chondrocytes may be regulated by a network of TGs and not require transamidation activity (164). In addition, TG2-modified calgranulin/S100A11 may trigger abnormal maturation of articular chondrocytes (54). Because hypertrophic differentiation of articular chondrocytes is proposed as a mechanism that drives osteoarthritis, these findings implicate TGs in osteoarthritis. Indeed, severe knee osteoarthritis in a guinea pig model is associated with increased synovial levels of TG2, but not FXIIIa (143), and cartilage degradation in the mouse model of injury-induced ostearthritis is reduced in the absence of TG2 (274). These proteins may also contribute to inflammatory osteoarthritis by triggering illegitimate maturation of articular chondrocytes. These findings suggest that identification of downstream mediators of TG-dependent chondrogenic differentiation may lead to development of novel therapeutics to treat inflamed joints without affecting normal homeostasis in cartilaginous tissue.

2. Bone and osteoblast differentiation

Osteoblasts are bone cells responsible for production and secretion of the collagen type I (COL I)-based mineralized bone matrix. Osteoblast lineage differentiation is under the master control of the transcription factors RUNX2 and Osterix, which control MSC commitment to the osteogenic lineage and the preosteoblast-to-osteoblast transition, respectively (192, 237, 324). Preosteoblasts form a transient provisional fibronectin matrix (31), while mature osteoblasts deposit the permanent COL I matrix (250, 336, 337). The advanced stages of osteoblast maturation are characterized by expression of alkaline phosphatase (114, 115), which is essential for matrix mineralization and a characteristic feature of fully differentiated osteoblasts (237). The final stage of bone and osteoblast differentiation is osteoblast transformation into osteocytes, which serves a mechanosensory function in the bone matrix and controls bone remodeling via regulation of osteoblast and osteoclast function (237, 324). TG2 and FXIIIa are expressed during osteoblast differentiation from early stages through osteocyte formation (11, 12, 171, 257, 266). Maturation and matrix mineralization in cultured osteoblasts are accelerated by the expression of exogenous TGs, suggesting that TGs act as positive autocrine regulators of osteoblast differentiation. Indeed, TG inhibitors arrest osteoblast differentiation at a very early stage, dramatically decrease COL I and fibronectin deposition and matrix accumulation as well as alkaline phosphatase expression and matrix mineralization (11, 12). These results suggest that TGs are essential for osteoblast differentiation. However, the precise molecular mechanism of this regulation is not known. During differentiation, TG2 levels remain constant, and the TG2 protein is retained at the cell surface in a high-molecular-weight complex that has no detectable crosslinking activity but may possess signaling activity (11). It has been postulated that TG2 on the cell surface promotes cell adhesion and spreading (56, 111, 112, 129, 367, 381) and may regulate osteoblast differentiation via this mechanism. In addition, in cultures of mature osteoblasts supplemented with ATP, TG2 may regulate ECM mineralization by acting as an ATPase in the ECM (39, 256, 258, 374) and increasing phosphate levels for hydroxyapatite precipitation or via phosphate signaling to promote terminal differentiation of osteoblasts into osteocytes (402).

In early osteoblasts, cellular FXIIIa associates with the plasma membrane and appears to be required for microtubule stabilization and collagen trafficking and secretion (11), a function that likely involves TG-mediated crosslinking of detyrosinated tubulin to form homotypic or heterotypic polymers. Tubulin has been described as a TG sub-
strate in other systems (87). At the preosteoblastic stage, FXIIla is secreted as a latent proenzyme that is incorporated into fibronectin and COL I matrix fibrils. Matrix-associated FXIIla seems to be activated to stabilize the permanent fibronectin/COL I matrix, which in turn promotes osteoblast maturation (292). Expression, cellular localization, and secretion of FXIIla are strongly stimulated by extracellular COL I and MAPK signaling (292).

A lack of any obvious skeletal phenotype in mouse models lacking either TG2 or FXIIla suggests that these two TGs have complementary functions and may act to compensate for each other (266). In this respect, increased FXIIla, TG1, and TG3 expression in bone and tendons in TG2-knockout mice has been observed (86, 266, 273, 338).

3. Muscle and myoblasts

Formation of cardiac, smooth, and skeletal muscle involves differentiation of myoblasts from MSCs. Expression of the muscle-specific contractile myofibrillar proteins (e.g., myosin, actin) is characteristic of myoblast differentiation, as is myoblast fusion during skeletal muscle maturation (40). TG inhibitors interfere with proper myoblast fusion resulting in anomalous elongation of the fused cells, which is indicative of defective cytoskeletal stabilization (35). TG-mediated crosslinking of the myosin chain occurs in different muscles, and this may contribute to myoblast differentiation and elongation (63, 139, 174). Therefore, stabilization of cytoskeletal elements via TG-mediated crosslinking might be the mechanism of TG-dependent regulation of cell differentiation, which is especially pertinent in cells with reduced levels of ECM, such as muscle cells.

4. Tendons and ligaments

The role of TGs in differentiation of tenocytes has yet to be extensively investigated despite evidence of expression of TG1 and TG2 in normal tendons (273). In mice, TG2-knockout tendons are anatomically normal. However, further biomechanical tests and injury models are needed to test a role for TG2 in tendon stabilization and response to injury (273). Whether TG1 can compensate for loss of TG2 expression in tendons also remains to be determined. TG3 and TG5 have been found in tendons, and FXIIla expression is selectively increased in injured tendons. The source of FXIIla in injured tendons remains to be determined, but may be related to blood vessel injury. Interestingly, increased TG activity in tendocytes is associated with disease. TG2 and FXIIla activity are increased in tendocytes exposed to carboxymethyl-lysine collagen and high glucose levels, mimicking an AGE-rich environment and diabetes, respectively (284, 300). These findings suggest that increased TG crosslinking activity and crosslink formation may contribute to the stiffening and pathological calcification of tendons in diabetic patients (10, 235). Similarly, elevated TG2 level is observed in calcified ligamentum flava of the spine (393), which provides additional evidence of the role of TG2 in pathology-related calcification of tendons.

V. ROLE OF TG2 IN CANCER

Cancer is a disease of dysregulated cell growth and differentiation. Genetic and epigenetic changes induced in response to chronic stressors (chemicals, infections, environmental) can constitutively activate survival signaling cascades and render them independent of growth factors. In addition to genetic and epigenetic alterations, cancer cells require additional input (probably induced by tumor stroma) to gain metastatic competence and ability to survive in host environment. In this context, chronic inflammation is considered to be an important factor in cancer initiation and progression (364, 365). Because TG expression, particularly TG2, is frequently increased in response to inflammation (223, 224, 227), it is likely that TGs play a role in cancer. Several lines of evidence suggest the involvement of TGs during cancer development even though the molecular mechanisms remain controversial. TG2 expression is low in primary tumors, but TG2 level is increased in drug-resistant and metastatic tumors (240, 280, 309, 372, 394). These observations suggest that TG2 may support cancer progression (6, 42, 229, 239, 271). In the following section, we discuss evidence that TG2 expression programs inflammatory signaling pathways in epithelial cancer cells to confer drug resistance and metastatic phenotypes.

A. TG2, Inflammation, and Cancer

A role for inflammation in cancer is well documented (151, 364, 365). Moreover, many inflammatory cytokines including TGF-β, TNF-α, IL-1, and IL-6, which are secreted at tumor cells, are known TG2 inducers (199, 296, 333). Excessive deposition of collagen by fibroblasts is observed in tumors in the form of a desmoplastic response, which is responsible for the clinical presentation of the tumor as a lump or dense stroma (16, 376) (FIGURE 9). The desmoplastic response of tumors appears to require TG2-catalytic activity (FIGURE 9). Increased TG2 expression in response to inflammatory cytokines, such as TGF-β in fibroblasts and epithelial cancer cells, accelerates the synthesis and deposition of fibronectin and collagen, whereas extracellular TG2 crosslinks them to stabilize the ECM. TG2-catalyzed crosslinking of ECM proteins can result in adverse outcomes, including diabetic nephropathy, kidney scarring, and atherosclerosis (60, 167, 312), and probably plays a similar role in the tumor desmoplastic response (FIGURE 9). The desmoplastic response involves an interplay between the invading tumor cells and altered ECM (16), hence the TG2-mediated alteration of the ECM is likely to impact tumor cell behavior. For example, breast cancer progres-
sion requires collagen crosslinking, ECM stiffening, and increased focal adhesion formation (212), and TG2-mediated crosslinking may alter the stiffness of the ECM and thereby promote a malignant phenotype.

B. TG2, Drug Resistance, and Metastasis

Resistance to therapy and metastasis are hallmarks of advanced cancer. Identification of tumor-encoded genes that promote drug resistance and metastasis is an important goal, as these proteins may serve as cancer biomarkers and also offer targets for therapy.

EMT is a major pathway that governs cell behavior during cancer progression. EMT is an embryonic process that can be reactivated in adult tissues in response to epigenetic changes (347). EMT is considered to be an attempt of the host to control inflammation and heal damaged tissue; however, in pathological contexts this response can cause damage. Epithelial tumor cells undergoing EMT lose epithelial tight junction proteins including E-cadherin and gain the expression of mesenchymal markers such as SMA, FSP1, vimentin, fibronectin, and desmin (172, 243, 347). These cells are involved in intravasation, movement of cancer cells in the circulation, extravasation, and micrometastases formation.

TG2 activates FAK, Akt, and NF-κB signaling, which are known to induce cancer cell EMT (315, 369, 371) (FIGURE 9). Inhibition of TG2 expression suppresses and increased TG2 expression enhances invasiveness and resistance to chemotherapy (131, 145, 280, 309, 368, 395). In addition, microvesicles, shed by TG2 overexpressing cancer cells, can confer transformed characteristics (e.g., anchorage-independent growth and enhanced survival capability) on normal fibroblasts and epithelial cells (14). Although TG2 alone is not sufficient to transform fibroblasts, it is likely that it collaborates with other proteins to mediate transforming action of the cancer cell-derived microvesicles.

C. TG2, EMT, and Cancer Stem Cells

TG2 expression is associated with induction of EMT and acquisition of stem-cell phenotype (195–197, 315). For ex-
ample, mammospheres generated from TG2-overexpressing nontransformed mammary epithelial cells (MCF10A) differentiate into mammary glandlike structures including Muc1-positive luminal cells and integrin α6-positive basal cells in response to prolactin treatment (197). In addition, there is a correlation among TG2, EMT, and acquisition of a stem cell-like phenotype in ovarian cancer cells (51). Cancer stem cells exhibit intrinsic resistance to chemotherapy and treatment of MCF-7 breast cancer cells with doxorubicin selects a small subpopulation of cells with stem cell characteristics that express high levels of TG2 (44). These results suggest that TG2 induces EMT and other stem cell traits in cancer cells.

Some information is available regarding TG2-regulated signaling pathways that drive these processes. TG2 expression results in constitutive activation of FAK, Akt, and NF-κB signaling (182, 369, 371), all of which are involved in cancer progression (172, 243, 276, 347, 375). NF-κB plays a prominent role in cancer progression, stemming from its ability to activate pro-growth, pro-metastasis, and anti-apoptotic genes (89, 328). Recently, a novel feedback loop where TG2 activates NF-κB and NF-κB, in turn, drives TG2 expression was identified (41) (FIGURE 9). In cancer cells, this TG2/NF-κB feedback loop may be self-amplifying, because high TG2 expression and elevated NF-κB activity are frequently observed in late-stage cancers. Most efforts to inhibit NF-κB activation in cancer cells have focused on small molecules that block the IKK kinase activity. In view of the observation that TG2-induced activation of NF-κB is mediated through IKK-independent mechanism (198), these inhibitors may not be effective. High-level NF-κB would, in turn, confer resistance to cell death and promote EMT and metastasis (178, 187).

NF-κB collaborates with TG2 to regulate expression of EMT transcriptional regulators including Snail, Twist, Zeb1, and Zeb2 (196, 315). TG2, in complex with the p65 subunit of NF-κB, is recruited to the promoter of the SNAIL gene, to increase Snail levels (187). TG2 also interfaces with TGF-β, a potent inducer of EMT. In breast cancer cells, TGF-β induced EMT requires TG2 expression (196). These findings suggest that TG2 may be an important regulator in the TGFβ signaling pathway that is required for EMT, and that crosstalk between TG2 and NF-κB may promote metastatic competence.

HIF-1 is an important hypoxia-response transcription factor that is regulated by NF-κB (142, 169, 318) and TG2 (195, 196, 198, 315) and has an important role in cancer cells. TG2-expressing cells display high basal levels of HIF-1α expression even under normoxic conditions, and suppression of either TG2 or NF-κB (p65/RelA) reduces HIF-1α level. Chromatin immunoprecipitation studies reveal that TG2 forms a complex with p65/RelA and that the complex binds to the NF-κB binding site in the HIF-1α promoter (FIGURE 6B). Like NF-κB and TG2, HIF-1α regulates EMT-related signaling, including accumulation snail, twist, and Zeb1 (127). These results suggest that TG2-induced NF-κB activation regulates HIF-1α expression.

Taken together, these observations suggest that aberrant epigenetic regulation of TG2 expression in cancer cells can reprogram inflammatory signaling networks (NF-κB activation, expression of HIF-1α, Snail, Twist, and Zeb) that influence EMT and stemness to promote drug resistance and the metastatic phenotype (FIGURE 10). Doxorubicin-resistant breast cancer cells express high levels of TG2 associated with hypomethylation of TGM2 promoter, whereas in doxorubicin-sensitive cells, which show no detectable expression of TG2, the TGM2 promoter is hypermethylated (6). In addition, treatment of drug-sensitive cells with 5-azadC restores TG2 expression and reduces sensitivity to doxorubicin (6). These findings suggest that TG2 may be a promising anti-cancer therapeutic target, as in vivo silencing of TG2 by delivery of liposomal-siRNA inhibits
the growth and dissemination of pancreatic and ovarian cancer cells and renders them sensitive to chemotherapeutic drugs in a nude mouse model (145, 368).

VI. REGULATION OF TG2 EXPRESSION AND TG2-REGULATED TRANSCRIPTIONAL PROCESSES

TG2 is gaining increasing attention for its role as a transcriptional regulator. In this section, we focus on how TG2 expression is regulated and how TG2 regulates gene expression.

A. Control of TG2 Gene Expression

Expression of TG2 is mainly controlled at the transcriptional level. Retinoids are well-known inducers of TG2 expression (83, 253, 290), and induction of TG2 expression by retinoids is controlled by the retinoic acid receptors (RARs) and retinoid X receptors (RXRs) (FIGURE 11). RARs and RXRs belong to a superfamily of nuclear receptors that bind to DNA as dimers. A study by Nagy et al. (253) showed that retinoid-dependent induction of murine TG2 expression is mediated by a tripartite response element located in the TG2 gene promoter. This study demonstrated that retinoic acid response elements are present at −1720 bp (RRE-1) and −1731 bp (RRE-2) in the murine TG2 promoter. Accordingly, RAR-RXR heterodimer and RXR homodimer receptor complexes bind to the murine TG2 promoter to regulate retinoic acid-dependent induction. In addition to RAR and RXR binding, the murine TG2 promoter requires an additional cooperating segment of the promoter (HR-1) to mediate activation of TG2 expression by retinoic acid (253). In the first characterization of the TG2 promoter, Lu et al. (222) reported that a 1.74-kb DNA region at the 5′-flanking region of the human TG2 promoter did not contain retinoic acid response elements, as the luciferase reporter they used failed to show induction in response to treatment with retinoic acid. However, subsequent studies establish that human TG2 expression is increased by retinoids, and an unusual binding site present in the TG2 promoter is likely to mediate induction (24, 357, 401).

TG2 expression is essential for retinoic acid-induced differentiation of human neuroblastoma cells (322, 357). The myc oncoproteins block differentiation and promote proliferation of malignant cancer cells (287), and TG2 expression is repressed by N-myc in neuroblastoma cells and by c-Myc protein in breast cancer cells, which may contribute to cancer cell proliferation and migration owing to a lack of differentiation. N-myc appears to suppress TG2 expression by recruiting histone deacetylase 1 to the Sp1 sites on the TG2 gene promoter (218, 341). Also, TG2 expression is epigenetically regulated by hypomethylation of CpG islands in its promoter in chemoresistant breast cancer (6), non-small cell lung cancer (280), and glioma (93) cells.

Although TG2 expression is suppressed in some cancer cells, it is increased in others, especially in cells resistant to chemotherapy or isolated from metastatic sites. TG2 expression is elevated in pancreatic carcinoma (372), breast carcinoma (239), malignant melanoma (109), ovarian carcinoma (145), lung carcinoma (280), and glioblastoma (395). As outlined above, inflammation (201) and hypoxia (313) are important in cancer, and TG2 expression is up-regulated by these processes. Indeed, the TG2 promoter contains response elements involved in inflammatory anti-hypoxic signaling (118, 154, 199, 244).

Cytokines increase TG2 expression in neuronal cultures (216). Also, LPS stimulates TG2 expression in several mammalian cell models, and metastatic tumor antigen 1 facilitates LPS-induced TG2 expression (120). An IL-6 response element (−1190 bp) is present in the human TG2 promoter (83), and IL-6 treatment of HepG2 human hepatoblastoma cells (333), human macrophages (117), and other cell types (232, 271) increases TG2. In addition, an NF-κB binding site is located at −1328 bp in the human TG2 promoter. NF-κB binding to this sequence in the TG2 promoter is observed in CCL4-treated rats (244). TG2 expression also can be induced by treatment with TNF-α in HepG2 cells (199).

TGF-β1 increases TG2 expression in apoptotic hepatocytes (118), human dermal fibroblasts (296), and human retinal pigment epithelial cells (295). The murine TG2 promoter contains a TGF-β response element at −868 bp upstream of the transcription start site (298). TGF-β1 increases TG2 promoter activity in Mv1Lu cells, an epithelial-like cell line derived from normal mink lung. Interestingly, treatment with TGF-β1 inhibits TG2 promoter activity in MC3T3 murine pre-osteoblastic cell. Moreover, BMP2 and BMP4, which are members of the TGF-β superfamily, also reduce TG2 promoter activity (298), indicating that the role of TGF-β family members in modulation of TG2 gene expression is stimulus and cell type dependent.

HIF-1 increases TG2 expression (154), and promoter deletion analysis revealed a sequence at 367 bp upstream of the transcription start site in the TG2 promoter that is required for this action (154). A comparison of TG2 promoter sequences shows that the response element is conserved in the human, murine, and guinea pig TG2 promoters. TG2 expression is also increased in response to hypoxia or ischemia in several other models (107, 350). Indeed, TG2 mRNA and protein level are elevated after stroke in rats, mice, and gerbils (107, 149, 350).

Lu et al. (222) characterized a TATA box (−29 bp) and CAAT box (−96 bp) in the human TG2 gene promoter.
FIGURE 11. Regulatory elements of human TG2 gene expression (TGM2). Glucocorticoid response element (−1399 bp), NF-κB (−1338 bp), IL-6 response element (−1190 bp), AP-2 (−634 bp), HRE (−367 bp), AP-1 (−183 bp), CAAT box (−96 bp), GC box: Sp1-binding motifs (−54 bp, −43 bp, +59 bp, and +65 bp), TATA box (−29 bp), and NF-1 (+4 bp and +12 bp). TG2 gene expression is upregulated in response to inflammation and hypoxia. Human TG2 expression is upregulated by treatment with retinoic acid, and potential retinoic acid response elements [RAR and RXR] are located in the human TG2 promoter. [From Gundemir et al. (125a), with permission from Elsevier.]

Variant forms of TG2 are also produced. The full-length 687-amino acid form of TG2 is the most abundant; however, other shorter TG2 variants are produced. Fraij et al. (113) isolated a truncated 548-amino acid form of TG2 (TGase-H) in retinoic acid-treated human erythroleukemia cells that differs from full-length TG2 in the last 10 amino acids (GKALCSWSIC versus EKSVPLCILY) (113). The 5′ end of this short isoform is the same as that of the long form up to base 1747, but the sequence differs from base 1748 to the termination codon. The divergent sequence contained an intron-exon boundary (CTGGTAA), which suggests alternative splicing (113). Another study described a similar truncated 548 amino acid isoform in an Alzheimer’s disease brain (64) along with a 555 amino acid isoform with a COOH-terminal sequence of GKPCVTGAFVDRGLTTC instead of QTQPITCQPSTQPGFIPR (64). Injured rat spinal cords express a 640-amino acid isoform of TG2, with 29 different amino acids at the COOH terminus (105). Truncated forms are also present in cytokine-treated rat astrocytes (249), leukocytes, vascular smooth muscle cells, and endothelial cells (203). The common feature is the presence of variable sequences at the COOH terminus. Because these short isoforms lack residues at the COOH terminus, GTP binding in TG2 is often impaired (203). Although the mechanism by which these short isoforms are generated has yet to be understood, some may result from intron read-through (64, 105, 113, 203, 249). Further studies are needed to determine how these isoforms are generated and to identify their physiological function.

B. Transcriptional Regulation by TG2

TG2 transcriptionally regulates several important targets (FIGURE 12). For example, TG2 induces differentiation and neurite growth in SH-SY5Y cells via a mechanism that may involve cAMP signaling, as TG2 induces adenyl cyclase, increases cAMP level, and enhances CREB activity in SH-SY5Y cells (356). These responses require TG2 transamidating activity (356). TG2 also increases CREB activity by interacting with protein phosphatase 2, a protein that dephosphorylates and inactivates CREB. The TG2-mediated increase in CREB activity leads to increased MMP-2 expression (310). In addition, membrane recruitment of TG2 is increased during erythroid differentiation of K562 cells in response to trans-retinoic acid and is associated with activation of Akt. TG2 overexpression increases CREB phosphorylation, an effect that is abrogated in the presence of wortmannin, a phosphatidylinositol 3-kinase inhibitor, im-
TG2 signaling is also important in cancer. Elevated TG2 expression is associated with acquisition of TRAIL resistance in lung cancer cells and increased MMP-9 expression, while TG2-siRNA reduces MMP-9 expression and restores TRAIL sensitivity (214). TG2 knockdown also reduces cell attachment, migration and invasion, and secretion of MMP-9 and MMP-1 in A431-III cells, an invasive cancer cell line (59, 215). These studies indicated that increased TG2 expression increases MMP-9 expression, perhaps by activating NF-κB. This response is not uniformly observed, as other studies report that TG2 expression suppresses MMP-9 promoter activity and reduces MMP-9 level. This was associated with reduced jun/fos recruitment to AP1 transcription factor binding elements in the MMP-9 promoter (5). The reason for the different outcomes of these studies is not known.

The studies above focused on modulation of transcription by cytosolic TG2. However, nuclear TG2 also regulates transcription. Although the levels of TG2 in the nucleus are significantly lower than in the cytoplasm, the presence of nuclear TG2 is well-documented (67, 106, 126, 209, 320). The mechanism whereby TG2 translocates to the nucleus is not known. TG2 has two putative nuclear localization signals (NLSs) located at amino acids 259–263 and 597–602, respectively (236), and TG2 interacts with importin-α3 (288), which may mediate nuclear translocation. However, study of this process is complicated, as mutation of the NLS site at amino acids 259–263 also inactivates the transamidating function of TG2 which complicates interpretation. However, mutation of the putative NLS site at amino acids 597–602 does not prevent nuclear localization of TG2 (236). It has also been suggested that TG2 contains a putative NLS site at amino acids 259–263 also inactivates the transamidating function of TG2 which complicates interpretation. However, mutation of the putative NLS site at amino acids 597–602 does not prevent nuclear localization of TG2 (236). However, mutation of the putative NLS site at amino acids 259–263 also inactivates the transamidating function of TG2 which complicates interpretation. However, mutation of the putative NLS site at amino acids 597–602 does not prevent nuclear localization of TG2 (236). However, mutation of the putative NLS site at amino acids 259–263 also inactivates the transamidating function of TG2 which complicates interpretation. However, mutation of the putative NLS site at amino acids 597–602 does not prevent nuclear localization of TG2 (236).

TG2 nuclear accumulation is also stimulus dependent. Treatment of A375-S2 human melanocytic cells with sphingosine increases nuclear TG2 (334), and in astrocytes, growth factors cause nuclear TG2 accumulation (45). Increased nuclear TG2 is also observed in differentiating NB4 cells (22). Hypoxic stress is a critically important signal that increases nuclear TG2 in neurons (106), and nuclear TG2 accumulation occurs in neurons in mice after stroke and in stroke patients in the areas of infarction (107). Moreover, TG2 suppresses hypoxia-induced HIF-1α signaling in SH-SY5Y cells (106) and primary rat neurons (Gundemir and Johnson, unpublished data), and the suppression does not require TG2 transamidation activity (125, 126). Furthermore, both wild-type and transamidation-inactive TG2 forms attenuate ischemic cell death in both SH-SY5Y cells and primary neurons (106). After stroke, infarcts in mice overexpressing human TG2 selectively in neurons are much smaller than those in wild-type mice, and stroke-induced proapoptotic gene expression is attenuated (107). Thus, in neurons and neuronal cell models, hypoxia causes accumulation of TG2 in the nucleus and increases cell survival.

In contrast, TG2 plays a different role in epithelial cells. Kumar and Mehta (198) demonstrated formation of a TG2
complex with the p65/RelA subunit of NF-κB and binding of this complex to the HIF-1α promoter. This binding results in increased transcription and accumulation of HIF-1α protein, even under normal oxygen conditions. The increased HIF-1α in turn, stimulates transcription of genes encoding glycolytic proteins including GLUT-1, hexokinase II, and lactate dehydrogenase-A. The net impact is increased glucose uptake, lactate production, and reduced mitochondrial respiration (Kumar and Mehta, unpublished data).

There are also studies suggesting that TG2 is associated with neurodegeneration (13, 204, 385). TG2 expression and activity are increased in patients with neurodegenerative disorders including Alzheimer’s (163, 185), Huntington’s (179, 211), and Parkinson’s diseases (13). A recent study suggests that TG2 regulates transcription in Huntington’s disease patient brains (211), suppression of PGC-1α and cyt c mRNA levels are lower in mutant huntingtin-expressing striatal cells compared with wild-type cells (236), and treatment of the mutant huntingtin-expressing cells with a TG2 inhibitor or TG2 knockdown restores PGC-1α and cyt c mRNA level. Additionally, TG2-knockout mouse fibroblasts contain higher levels of cyt c than wild-type mouse fibroblasts. Furthermore, in the presence of mutant huntingtin, the interaction of TG2 with PGC-1α and cyt c promoters is increased, resulting in suppression of transcription. In these experiments, TG2 transamidation activity is necessary for suppression of promoter activity. Considering the fact that TG2 expression is increased in Huntington disease patient brains (211), suppression of PGC-1α and cyt c transcription by TG2 in mutant huntingtin expressing cells may contribute to disease progression.

TG2 accumulates in the nucleus in ethanol exposed rat hepatocytes (57). Gene expression analysis reveals that ethanol treatment reduces c-Met expression to a greater extent in TG2 wild-type hepatocytes compared with TG2 knockout mouse hepatocytes. c-Met is the receptor for hepatocyte growth factor and is involved in liver regeneration (144). This is associated with TG2 crosslinking of Sp1 which is required for c-Met expression (339). This is consistent with patient data, as TG2-mediated Sp1 crosslinking is observed in liver from patients with alcohol-related liver disease (384). In addition to increasing the expression of TG2, treatment with ethanol appears to increase TG2 transamination activity (339, 388). These findings indicate that nuclear TG2 can modulate gene transcription in response to ethanol. However, it is not known whether this effect requires TG2 transamination activity.

VII. PERSPECTIVES AND FUTURE DIRECTIONS

Despite a growing understanding of the biological functions of TGs and their mechanism of action, many questions remain unanswered in a host of cell types. Several areas that would benefit from further study are outlined below. Some of these areas are highly relevant to disease. For example, while the role of transglutaminases in differentiation is established, efforts are only beginning to address the role of these enzymes in the stem cell niche and the role of TGs in stem and progenitor cells. This area has important implications for tissue renewal and cancer cell survival. Diabetes is also an important area that would benefit from additional studies. Knowledge is limited regarding the role of TG2 in diabetes and in adipocyte differentiation. In this context, FXIIa was recently identified as a top obesity gene in a genome-wide screen for single nucleotide polymorphism linked to basal metabolic index. The significant association of FXIIIa with obesity was further confirmed in a large European ENGAGE consortium study of more than 21,000 unrelated individuals as well as in the GenMets cohort study (261a). The interplay among various TG forms is another area that is important in the context of disease. The compensatory response following loss of TG2 in specific tissues requires more detailed analysis. There is now substantial evidence for compensation by other TG forms, but more needs to be done. This analysis will advance our understanding of the biological functions of TG-mediated crosslinking in different tissues and may also offer focused approaches to target TG in various diseases. The area of inhibitors of transglutaminase is also an area for additional investment, as it would be extremely useful to have compounds that specifically inhibit each of the TG forms.

Understanding the role of various TGs in tissues that express multiple TG enzymes is also an important arena. An example is monocyte differentiation. TG2 and FXIIIa partially translocate to the nucleus in differentiating monocytes (22, 352), and both enzymes affect expression of a large number of genes (72, 352). Identification of TG2- and FXIIIa-responsive genes may be informative regarding the role of these proteins in monocyte differentiation. This is also an issue in many other tissues. New knowledge would also be useful regarding the role of transglutaminases in other immune cells. TGs may indirectly regulate inflammatory responses via activation of macrophages, natural killer cells, and antigen-specific cytotoxic T lymphocytes. TG2, for example, causes epithelial cells to secrete IL-6 (272), which is likely to promote immune cell inversion.

Another important area is the role of TGs in cancer. It is interesting that elevated expression of TG2 confers drug resistance. However, we have a long way to go before we understand the mechanism. A systematic study of a large cohort of patients should be performed to determine whether TG2 is a valid prognostic marker. Similarly, TG2-induced activation of NF-κB and accumulation of HIF-1α represent interesting areas for future research. Constitutive activation of NF-κB is linked to etiology of various chronic inflammatory conditions, including cancer. Understanding of process whereby TG2 regulates NF-κB activa-
tion may yield ways to block this pathway and mitigate disease. Similarly, inhibition of TG2-mediated increase in HIF-1α accumulation may be relevant in cancer therapy, as HIF-1α activation is a major pathway whereby cancer cells influence glucose metabolism and aerobic glycolysis (88).

A remarkable feature of these studies is that no more than two decades ago transglutaminases were thought to function solely as enzymes that covalently crosslinked proteins to assemble barriers. The scope of this review points to the vast amount of new knowledge that has accumulated in the past two decades which point to a pivotal role for these proteins in regulating cell homeostasis.

ACKNOWLEDGMENTS

We thank Kathleen Reinecke for persistent, patient, and expert assistance in the preparation of this manuscript. We also thank Donald R. Norwood for critical reading of and editorial help with this review and Dr. Soner Gundemir for the helpful discussion. We also thank Drs. Candace Kerr and Ellen Rorke for critically reading the manuscript.

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GRANTS

This work was supported by National Institutes of Health Grants AR053851 and CA131974 (to R. L. Eckert); AR057126, HL093305, and DK071920 (to M. Nurmins-kaya); and NS0065825 (to G. Johnson); a grant from the Bayer Healthcare System (Grants4Targets) and SK Agarwal donor funds (to K. Mehta); Canadian Institutes of Health Research Grants MOP-119103, NHG-107768, and IMH-407Physiol Rev • VOL 94 • APRIL 2014 • www.prv.org

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