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IN PERSPECTIVE

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Transglutaminase 2 takes center stage as a cancer cell survival factor and therapy target

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Transglutaminase 2 (TG2) has emerged as a key cancer cell survival factor that drives epithelial to mesenchymal transition, angiogenesis, metastasis, inflammation, drug resistance, cancer stem cell survival and stemness, and invasion and migration. TG2 can exist in a GTP-bound signaling-active conformation or in a transamidase-active conformation. The GTP bound conformation of TG2 contributes to cell survival and the transamidase conformation can contribute to cell survival or death. We present evidence suggesting that TG2 has a role in human cancer, summarize what is known about the TG2 mechanism of action in a range of cancer types, and discuss TG2 as a cancer therapy target.

KEYWORDS
apoptosis, cancer, cancer stem cell, cancer therapy, NC9, signaling, transglutaminase inhibitor, transglutaminase 2

1 | INTRODUCTION

Although cancer therapy has improved greatly in recent years, drug resistance, tumor recurrence and metastasis are still important problems. Thus, there remains a pressing need for new therapeutic options. This requires that new therapy targets be identified. Transglutaminase 2 (TG2) has emerged as a key cancer cell survival factor in a wide range of cancer cell types. TG2 was discovered in the 1950's based on its ability to catalyze incorporation of low-molecular-weight primary amines onto proteins.[1] This transamidase (TGase) enzymatic function identified TG2 as involved in protein trans-amidation and assembly of macromolecular complexes held together by ε-(γ-glutamyl)lysine isopeptide bonds.[2] The first glimmer of evidence that TG2 may have activity beyond functioning as a transamidase came with the discovery that TG2 is a GTP/GDP binding protein that hydrolyzes GTP and acts as a G protein.[3] This discovery opened the floodgates leading to the assignment of TG2 as a key cancer cell survival factor that triggers various signaling programs to drive epithelial mesenchymal transition (EMT), cancer stem cell survival, angiogenesis, drug resistance, inflammation, and metastasis. The present review explores the role of TG2 by cancer type. Although the details are complicated, and there are exceptions, the literature supports the idea that TG2, in the GTP-bound/closed/signaling-active conformation, drives cancer cell and cancer stem cell survival. In contrast, the open/extended/TGase-active conformation can enhance cancer cell survival or drive cell death in a context-dependent manner.

2 | TRANSGLUTAMINASE 2

Because of its recognized role in disease and wide tissue distribution, TG2 is the most extensively studied transglutaminase family member.[2,4,5] TG2 has an amino-terminal β-sandwich domain which binds fibronectin and integrins, a catalytic core that contains the TGase catalytic triad and two carboxyl-terminal β-barrel domains which contain GTP/GDP and phospholipase C binding sites (Figure 1).[2] TG2 has both signaling and enzymatic functions that are mutually exclusive and dependent upon protein conformation. TG2 has been described as a "Swiss Army Knife" that can exist as a GTP-bound/closed/folded/signaling-active conformation and as a calcium-bound/open/extended

Abbreviations: EMT, epithelial mesenchymal transition; TG2, transglutaminase 2; CHIP, carboxyl-terminus of hsp70-interacting protein.

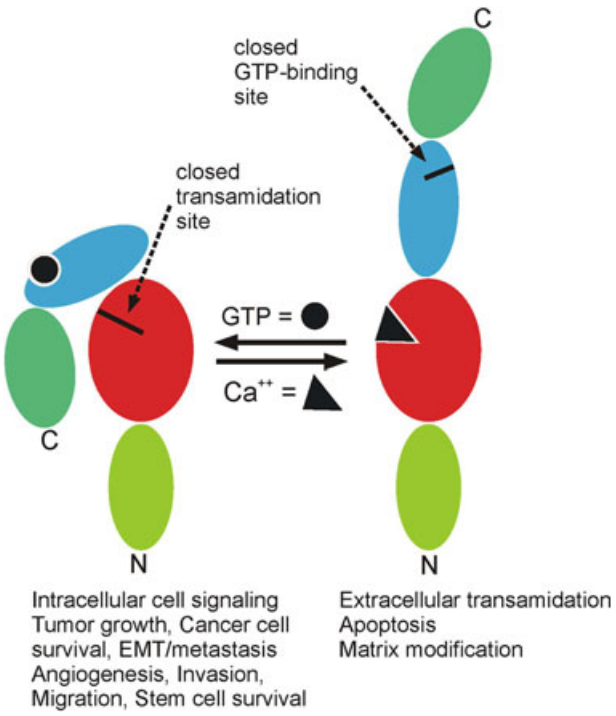

FIGURE 1 Transglutaminase 2 structure and function relationships. The transglutaminase amino-terminal β-sandwich domain (light green), a transamidase domain (TGase)(red), the GTP/GDP binding domain (blue), and the C-terminal β-barrel domain (green). TG2 can exist in two mutually and functionally exclusive conformations. Intracellular GTP/GDP binding to the TG2 binding domain causes TG2 to assume a closed/folded/signaling-active GTP-bound conformation which drives intracellular signaling. In contrast, when exposed to elevated free calcium levels in the extracellular environment, or a stimulus-dependent increase in intracellular calcium, TG2 assumes the open/extended transamidase-active conformation[6,7] that functions as a transamidase. Note that activity in the protein is mutually restricted —when GTP/GDP is bound, the transamidase domain is closed, and when calcium is bound, the GTP/GDP binding domain is closed [Color figure can be viewed at wileyonlinelibrary.com]

transamidase-active conformation.[4] The closed GTP bound conformation is dominant in the intracellular environment where GTP levels are elevated,[6,7] while the open highly extended transamidase-active (TGase) conformation is favored in the extracellular environment and in intracellular areas of the cell where calcium levels are elevated. Thus, in the presence of GTP/GDP TG2 flips closed and in the presence of calcium it flips open. An important feature is that the activities of the protein are mutually exclusive: the transamidase site is inactivated in the GTP bound form and GTP binding is inactivated in the calcium-bound form.

We will also discuss several other members of the transglutaminase family that have been implicated as having a role in cancer. These include transglutaminases 1, 3, 4, 5, and 6 (TG1, TG3, TG4, TG5, TG6). TG1, TG3, and TG5 are typically expressed in the epidermis and other stratified tissues, TG4 is selectively expressed in the prostate, and TG6 is expressed in testis, lung, and brain.[2] In common with TG2,[8] TG3 and TG5 possess both GTP binding and TGase (transamidase) domains.[9,10] In contrast, TG1, TG4, and TG6 encode a TGase domain, but lack a GTP binding domain.[2]

TG2 levels are increased in cancer cells and tissues in leukemia,[11] breast cancer,[12] ovarian cancer,[13] prostate cancer,[14] lung cancer,[15] glioblastoma,[16] renal cancer,[17] epidermal squamous cell carcinoma,[18] pancreatic cancer,[19] cervical cancer,[20] esophageal adenocarcinoma,[21] oral squamous cell carcinoma,[22,23] mesothelioma,[24] gastric cancer,[25] and colon cancer.[26] Moreover, TG2 levels are further enriched in cancer stem cells where it functions to maintain the cancer stem cell phenotype.[27,28] In addition, TG2 expression level in tumors correlates directly with enhanced metastasis, shorter cancer-free survival, chemotherapy resistance and poor patient outcome.[11,12,29,30] TG2 structure-function and interaction with other proteins[2–4,8,31–34] and role in enhancing cancer stem cell survival[18,27] have been discussed in recent reviews. This review will highlight the role of TG2 in cancer and discuss TG2-regulated pathways that are implicated in cancer progression in various cancer cell/tumor types. When relevant, other transglutaminases are also mentioned. As will become clear from this survey, the most important issue regarding the role(s) of TG2 in cancer, is the fact that TG2 appears in two mutually exclusive flavors—a GTP-bound/closed/folded/signaling-active conformation and a calcium-bound/open/extended transamidase-active conformation (Figure 1).[4,6,7] We will refer to the GTP/GDP binding activity as TG2 GTP binding activity, and the TG2 transamidase activity as TG2 TGase activity.

3 | TG2 IN TUMOR TYPES

3.1 | Gastric cancer

Gastric cancer is an extremely malignant disease due to its rapid progression, tendency to metastasize, and resistance to chemotherapy.[35] TG2 is highly expressed in human gastric tumors and expression is positively correlated with tumor stage.[25] TG2 promotes gastric cancer cell proliferation, migration, invasion, tumor formation, and peritoneal metastasis via a mechanism that requires ERK1/2 activity[25] (Figure 2A). In addition, GX1, a cyclic 7-mer peptide that inhibits gastric cancer by suppressing neovascularization, binds to TG2 in tumor-associated vascular endothelial cells, to reduce TG2 GTP binding activity and suppresses NF-κB and HIF-1α signaling. Thus, TG2 has an important role in both tumor cells and supporting vasculature, and the peptide inhibitor data suggests that TG2 GTP binding activity is required for this pro-cancer action[36] (Figure 2A).

Photodynamic therapy is an important cancer treatment modality which induces reactive oxygen species production and increases intracellular calcium level. In gastric cancer cells, photodynamic therapy increases TG2 TGase activity and apoptotic cell death[37] (Figure 2A). TG2 TGase activity appears to be important for this death response, as TG2 knockdown or treatment with BAPTA-AM (to chelate intracellular calcium) reduces apoptosis and cell death in response to photodynamic therapy.[37] This suggests that photodynamic therapy induces reactive oxygen species production leading to

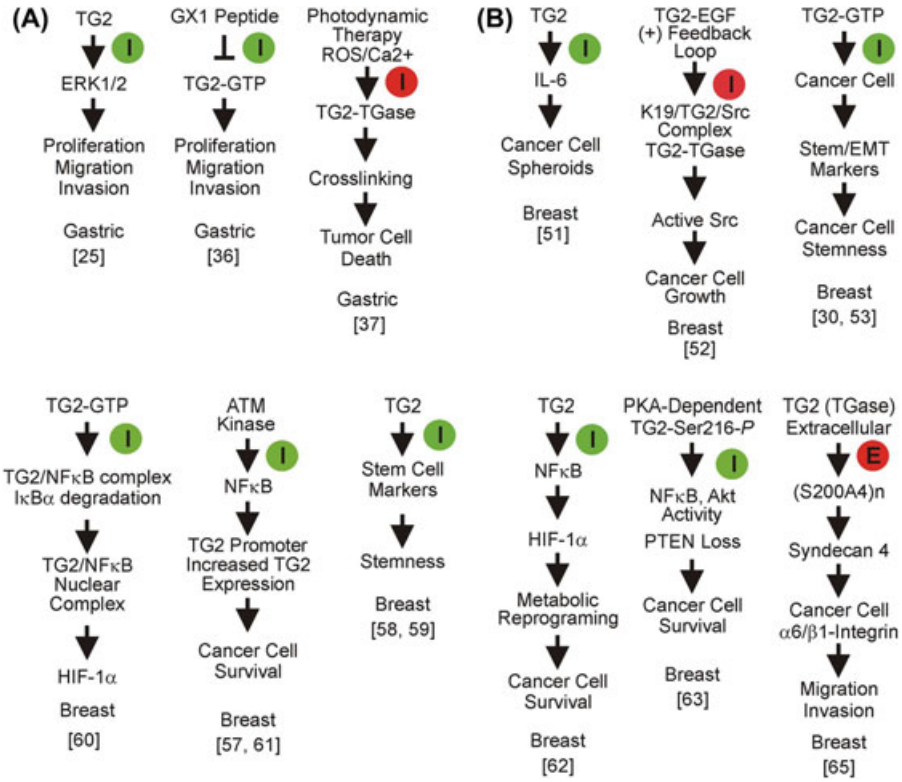

FIGURE 2 Transglutaminase signaling in gastric and breast cancer. The arrows indicate positive activation. The green circle indicates a likely mechanism mediated via the TG2 GTP binding domain and red circles indicate events mediated via the TG2 TGase activity. "I" indicates an intracellular mechanism and "E" indicates extracellular. "*P*" indicates the target is phosphorylated. "TG2-GTP" indicates that the GTP binding domain has been strongly implicated in mediating the regulation. A, The gastric cancer references are.[25,36,37] B, The breast cancer references are.[30,51,52,53,57–63,65] [Color figure can be viewed at wileyonlinelibrary.com]

release of intracellular calcium which binds to and activates TG2 TGase activity leading to cell death. Thus, photodynamic therapy is an example of a treatment that elevates intracellular calcium to shift TG2 to the open TGase-active/GTP binding-inactive form (Figure 1), thereby shifting the protein from GTP-powered survival mode to TGase-powered cell death mode. As part of this process, TG2 TGase activity contributes to calpain activation, Bax translocation and apoptosis-inducing-factor release leading to apoptotic cell death.[38,39]

Although direct measurements of TG2 intracellular conformation have not been performed in unperturbed gastric cancer cells, indirect data suggests that TG2 exists in the closed GTP bound form in these cells where it is expected to drive cancer cell survival signaling[25,36] (Figure 2A). This suggests that TG2 inhibitors, which suppress GTP binding by indirectly disordering the TG2 GTP binding site,[6,40–42] may be a useful treatment for gastric cancer.

TG1 has also been studied in gastric cancer.[43] TG1 expression is increased in patient tumors and in cultured gastric cancer cells, and TG1 knockdown inhibits cell proliferation, enhances apoptosis, and increases gastric cancer cell susceptibility to chemotherapeutic agents. Mechanistic studies indicate that TG1 activates Wnt signaling which is reduced in TG1 knockdown cells. These surprising results support a possible role for TG1 in gastric cancer cell survival.[43] These studies are unique, as they suggest that TG1, a family member that lacks the GTP binding domain, can drive cancer cell survival.

3.2 | Oral squamous cell carcinoma

TG2 is a biomarker of oral squamous cell carcinoma, as mRNA microarray analysis shows elevated TG2 mRNA in primary tumors and metastasis.[22] Moreover, immunostaining of 56 oral tumor samples, suggest that elevated TG2 level predicts enhanced tumorigenesis and poor prognosis.[23]

TG3 may have a tumor suppressor role in oral cancer, as proteomic analysis reveals a marked reduction in TG3 levels in oral cancer samples due to hypermethylation of the TG3 gene locus.[44] TG3 TGase activity is involved in crosslinking precursor structural proteins to facilitate terminal cell differentiation in stratified epithelia,[45–47] and so loss of TG3 would be consistent with reduced cell differentiation during cancer progression.[44] However, TG3 also possesses GTP binding activity[9] and so TG3 loss may also modify cell signaling in a way that permits tumor formation. Further mechanistic studies would be helpful in understanding the role of TG2 and TG3 in oral cancer.

3.3 | Breast cancer

TG2 is elevated in both tumor cells and surrounding stroma in breast cancer. TG2 expression in breast tumor stroma is associated with reduced disease-free survival and increased risk of cancer recurrence.[48,49] Elevated TG2 in ER-negative breast tumor cells indicates a

poor prognosis and enhanced lymph node metastasis[50] and high TG2 level, detected by tissue microarray, is associated with tumor recurrence, elevated IL-6 expression, and reduced metastasis-free survival[51] (Figure 2B).

A range of cell signaling studies have been performed to understand how TG2 drives cancer cell survival and tumor formation. Epidermal growth factor (EGF) signaling is important for survival and growth of breast cancer cells, in part, by impacting TG2 function.[52] TG2 knockdown or treatment with TG2 inhibitor blocks EGF-stimulated anchorage independent breast cancer cell proliferation.[52] As part of this mechanism, EGF-stimulated Ras and Cdc42 signaling, activates PI3K and NFκB to increase TG2 level, implying a positive feedback loop. In addition, overexpression of wild-type TG2, but not TGase activity-inactive TG2, mimics the growth advantages afforded by EGF. The authors propose that TG2 forms a complex with keratin 19 and src and that TGase activity modifies keratin 19 to activate src signaling and enhance cancer cell survival[52] (Figure 2B). Thus, it appears that TG2 TGase modification of K19 is necessary to form a scaffold that activates Src signaling. These findings are in contrast to other studies which suggest that the TG2 GTP binding activity, but not TG2 TGase activity, is required for breast cancer cell survival[30,53] (Figure 2B).

Resistance is an important response to treatment with anti-cancer drugs and TG2 has been proposed to be a drug-resistance factor. TG2 knockdown sensitizes cells to docetaxel.[54] TG2 level is increased in breast cancer cells following treatment with rapamycin, an mTOR (mechanistic target of rapamycin complex 1) inhibitor[55] and TG2 knockdown renders these cells hypersensitive to rapamycin.[55] TG2 also mediates resistance to histone deacetylase (HDAC) inhibitors. For example, treatment with vorinostat, a clinically approved HDAC inhibitor, selects resistant cells that express elevated TG2, and co-treatment of with TG2 inhibitor restores vorinostat sensitivity.[56] TG2 knockdown also restores breast cancer cell sensitivity to doxorubicin.[57] Thus, TG2 has emerged as an important breast cancer drug-resistance factor.

TG2 and NFκB are important regulators in breast cancer. TG2 promotes epithelial mesenchymal transition (EMT) in breast cancer cells, which is associated with loss of E-cadherin and increased expression of Snail, Zeb, Zeb2 and Twist, and increased stem cell marker expression[58,59] (Figure 2B). These events involve NFκB binding to the HIF-1α promoter to increase HIF-1α gene expression, which then stimulates expression of Zeb1, Zeb2, Snail, and Twist[60] (Figure 2B). TG2 activation of NFκB is mediated via an unconventional interaction between TG2 and IκBα, the NFκB signaling inhibitor, leading to IκBα degradation. Simultaneously, TG2 and NFκB (p65-RelA) form a nuclear complex that binds to and activates the HIF-1α promoter. Both wild-type TG2 and TGase activity-inactive TG2 mutants stimulate NFκB leading to HIF-1α induction, suggesting that the regulation requires the TG2 GTP-binding domain.[60] Moreover, ATM kinase drives TG2 expression and a TG2/NFκB positive feedback loop has been identified where NFκB (p65-RelA) interacts with binding sites within the TG2 gene promoter to activate TG2 expression which, in turn, further activates NFκB[57,61] (Figure 2B). TGFβ is a key regulator of EMT, but it fails to stimulate EMT in cells lacking TG2, suggesting TG2 may be a downstream mediator of TGFβ action[58] (Figure 2B). TG2 activation of NFκB, leading to increased HIF-1α gene expression, also stimulates glucose uptake and lactate production, and decreases mitochondrial oxygen consumption, while suppression of TG2, NFκB (p65-RelA) or HIF-1α attenuates these changes [62] (Figure 2B). Covalent modification of TG2 can also influence TG2 regulation of NFκB. For example, protein kinase A phosphorylation of TG2 at serine-216 precedes NFκB activation and PTEN downregulation in MCF-7 and T47D cells[63] (Figure 2B).

TG2 also drives IL-6 cytokine production in breast cancer cells, and TG2 or IL-6 loss reduces tumor xenograft growth and metastasis.[51] Moreover, IL-1β induces IL-6 production in TG2 overexpressing MCF-7 cells via a mechanism that requires NFκB, PI3K, and JNK, and leads to enhanced stemness, invasion, survival, and tumor growth, while suppressing IL-6 or IL-1β production attenuates these events.[64]

The above studies describe an intracellular role for TG2, but it also impacts extracellular events. S100A4 is an S100 family protein that mediates tumor cell migration. S100A4 overexpression in rat mammary cells results in highly migratory phenotype that is suppressed by TG2 knockdown or TG2 inhibitor treatment.[65] At the mechanistic level, TG2 inhibitor (R294) treatment of MDA-MB-231 breast cancer cells reduces TG2 TGase-dependent S100A4 multimer formation[65] (Figure 2B). The authors propose that extracellular TG2 crosslinks S100A4 to form a multimer that binds syndecan-4 to activate syndecan-4 dependent α5β1-integrin outside-in signaling to stimulate tumor cell migration and invasion.[65] In this model, TG2 TGase activity stimulates the cancer phenotype. These finding are consistent with other studies showing that S100 proteins are transglutaminase substrates.[66–68]

3.4 | Ovarian cancer

Elevated TG2 was detected in a sample of 93 human ovarian tumors and ascites fluid.[69] In a mice harboring OV90 cell orthotopic tumors, administration of recombinant TG2 in the peritoneal fluid enhanced metastatic cancer cell implantation on the omentum, mesentery, and abdominal flanks. A similar increase in dissemination was observed for OV90 cells engineered to overexpress TG2, and was associated with increased cell adhesion to collagen[69] (Figure 3A). Treatment of cells with recombinant wild-type TG2 induced non-canonical NFκB signaling, but this was not observed with recombinant TG2(C277A), a TGase activity-inactive TG2 mutant, suggesting that TG2 TGase activity is required for malignant spread.

TG2 also physically associates with and recruits Src to phosphorylate β-catenin, leading to release of β-catenin from E-cadherin thereby permitting E-cadherin to activate gene expression[70] (Figure 3A). Moreover, TGFβ, which is secreted in the ovarian cancer microenvironment, induces TG2 to drive spheroid formation, ovarian cancer cell metastasis, and EMT.[71] These events require interaction of TG2 with fibronectin, as inhibiting the TG2/fibronectin interaction reduces stability of the TGFβ/fibronectin/TG2 complex to reduce adhesion and biological response.[72]

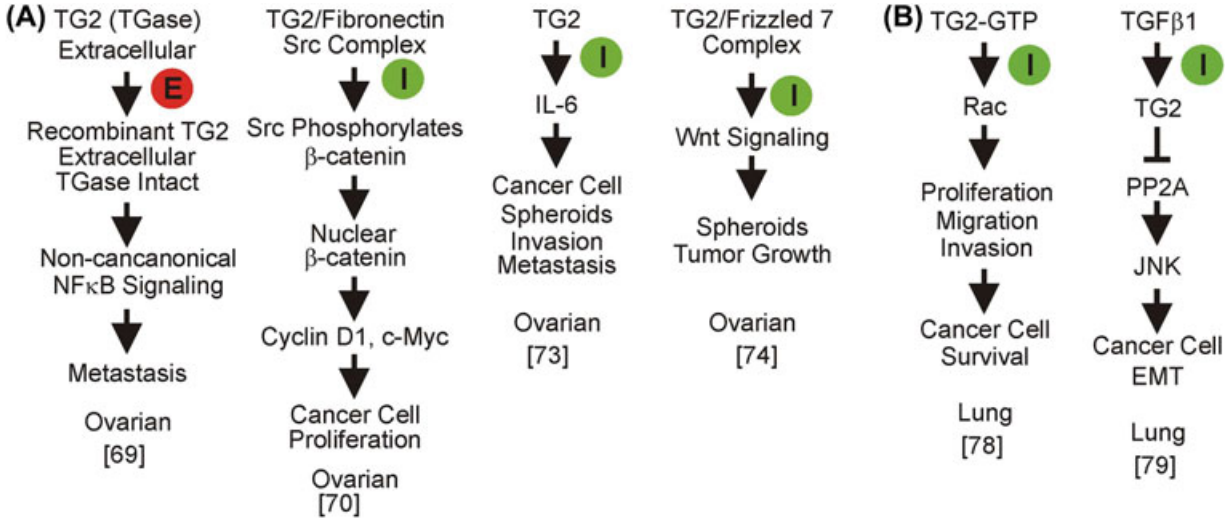

FIGURE 3 Transglutaminase signaling in ovarian and lung cancer. The conventions are as indicated in Figure 1. A, The ovarian cancer reference are.[69,70,73,74] B, The lung cancer reference are.[78,79] [Color figure can be viewed at wileyonlinelibrary.com]

Other TG2-dependent events also enhance ovarian cancer cell survival and metastasis, including TG2-dependent IL-6 production to drive tumor sphere formation[73] (Figure 3A) and formation of a TG2/Frizzled 7 complex to drive Wnt/β-catenin signaling[74] (Figure 3A). Thus, TG2 impacts NFκB, IL-6, TGFβ, and Wnt/β-catenin signaling in ovarian cancer cells.

3.5 | Lung cancer

Two independent studies of non-small cell lung cancer patients show that elevated TG2 level predicts disease recurrence and shorter disease free survival, and suggest that TG2 level may be a prognostic marker of disease progression.[75,76] Moreover, in chemotherapy patients, progression free survival is lengthened if the patients have low tumor levels of TG2.[15] Other studies also support a role for TG2 in this disease. For example, treatment with the TG2 inhibitor, KCC009, enhances lung cancer cell radio-sensitivity via a mechanism that involves restoration of p53 function.[77] Moreover, TG2 expression correlates with lung cancer cell invasion and migration, and TG2 TGase activity is not required, since expression of the TGase-deficient mutant, TG2(C277S), maintains cancer cell migration and invasion[78] (Figure 3B). This suggests that the TG2 GTP binding domain is required for cancer cell invasion and migration. In A549 lung cancer cells, TG2 knockdown or pharmacologic suppression of JNK activity, reduces TGFβ1-induced EMT, and additional studies suggest that TG2 activates JNK, via suppression of PP2A activity[79] (Figure 3B). TG2 also appears to influence drug sensitivity in these tumors, as treatment with TG2 inhibitor or TG2 knockdown restores TRAIL-induced apoptosis.[80,81]

3.6 | Pancreatic cancer

Pancreatic cancer is an extremely aggressive and therapy resistant disease. Screening of the cancer genome atlas indicates a correlation between high TG2 expression and reduced overall patient survival.[82] TG2 knockdown in pancreatic ductal adenocarcinoma cells reduces xenograft growth and enhances sensitivity to gemcitabine, and it has been suggested that TG2 released from pancreatic tumor cells activates fibroblasts in the tumor microenvironment to produce laminin which protects the cancer cells by constructing a dense desmoplastic stroma.[82] Studies of TG2 released from pancreatic tumor cells shows that secreted TG2 modifies the stroma by cross-linking collagen which activates fibroblasts and that this "stiff" matrix activates YAP1/TAZ signaling in the pancreatic cancer cells to drive tumor formation [83] (Figure 4A). This is consistent with studies in squamous cell carcinoma which showing that TG2 targets YAP1/TAZ signaling to drive cancer cell survival.[28,84,85]

Eukaryotic elongation factor-2 kinase (eEF2K) is a negative regulator of protein elongation that acts via phosphorylation of eukaryotic elongation factor 2 (eEF2), and is a key component of the translation machinery.[86] eEF2K is overexpressed in pancreatic cancer cells and is associated with elevated TG2 level[86] (Figure 4A). Moreover, knockdown of eEF2K reduces cell invasion and migration, and reduces TG2 mRNA and protein level. TG2 appears to have a role in regulating downstream signaling events, as knockdown of either eEF2K, or TG2, reduces Snail and ZEB1 level leading to reduced EMT[86] (Figure 4A) Thus, eukaryotic elongation factor-2 kinase appears to utilize TG2 to enhance pancreatic cancer cell migration, invasion and EMT. TG2 also regulates pancreatic cell morphology, since TG2 silencing reduces the 2-O-tetradecanoylphorbol-13-acetate dependent phosphorylation of keratin 8, and suppresses 2-O-tetradecanoylphorbol-13-acetate dependent keratin filament reorganization in PANC-1 cells.[87] These studies argue for a central role for both intracellular and extracellular TG2 in the pathogenesis of pancreatic cancer.

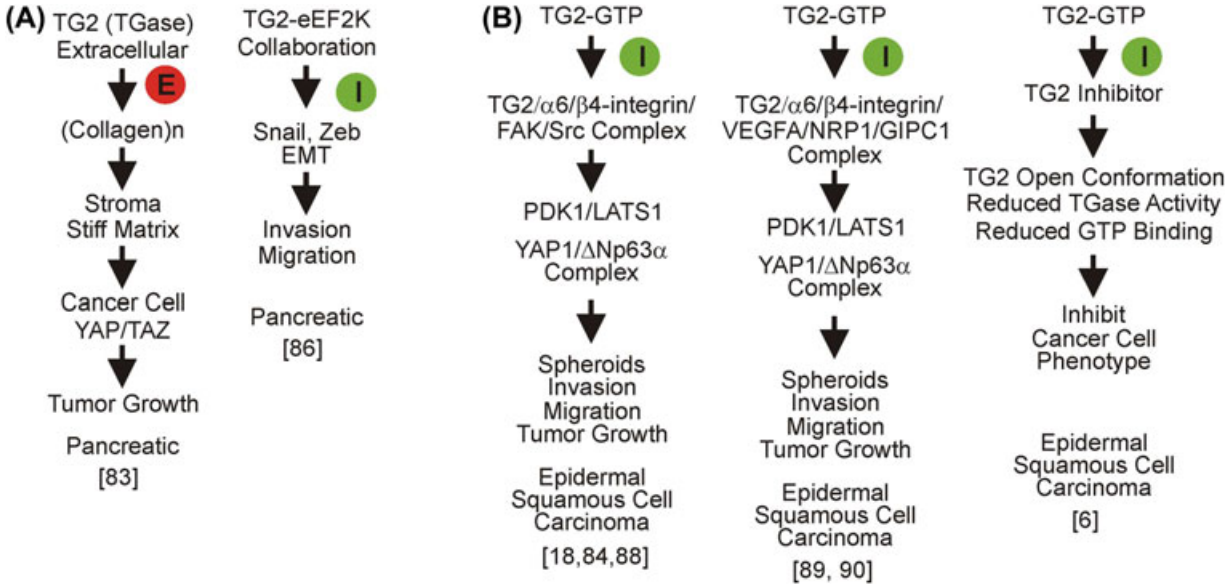

FIGURE 4 Transglutaminase signaling in pancreatic cancer and epidermal squamous cell carcinoma. The conventions are as indicated in Figure 1 and the flat head arrows indicate inhibition. A, The pancreatic cancer references are.[83,86] B, The squamous cell carcinoma references are.[6,18,84,88,89,90] [Color figure can be viewed at wileyonlinelibrary.com]

3.7 | Epidermal squamous cell carcinoma

TG2 is highly elevated in epidermal cancer stem cells (ECS cells) and TG2 knockdown or suppression of TG2 function with TG2 inhibitor (NC9) reduces ECS cell survival, spheroid formation, matrigel invasion, migration, and EMT[18,84] (Figure 4B). This reduction in stem cell phenotype is associated with activation of apoptosis, and TG2 mutant studies reveal that TG2 GTP-binding activity, but not TGase activity, is required to maintain the ECS cell phenotype[18,84] (Figure 4B).

Interestingly, TG2 action in ECS cells does not involve activation of NF-κB signaling.[18,84] Instead, TG2 activates YAP1/ΔNp63α signaling to drive cancer stem cell survival and tumor formation.[88] In this cascade TG2 interacts with α6/β4-integrin to stimulate FAK/Src signaling leading to PI3K activation of PDK1. PDK1, in turn, inhibits hippo signaling leading to enhanced nuclear YAP1 accumulation which forms a stable YAP1/ΔNp63α nuclear complex that drives the cancer stem cell phenotype[88] (Figure 4B). As noted above, TG2 forms a complex with α6/β4-integrins to activate YAP1/ΔNp63α signaling in skin cancer cells. Additional studies show that the NRP-1 angiogenesis mediator and GIPC1, a scaffolding protein, participate in a related complex to drive angiogenic processes in squamous cell carcinoma[89,90] (Figure 4B). In this cascade, VEGF-A forms a complex with NRP-1, α6/β4-integrin, GIPC1 and TG2 to trigger signaling events that activate YAP1/ΔNp63α signaling. Since GIPC1 can couple to a large number of downstream cascades, this suggests that this TG2 inclusive complex may activate many downstream cancer survival pathways[89,90] (Figure 4B). An example is a recent manuscript showing that VEGFA/NRP-1 activates GIPC1/Syx complex formation to activate RhoA/Rock signaling and p38 MAPK activity to drive epidermal cancer stem cell survival.[91]

Studies using ECS cells establish the intracellular mechanism of action of several transglutaminase 2 inhibitors, including NC9, VA4, VA5, and CP4d[6] (Figure 4B). NC9, VA4, and VA5 are irreversible and CP4d is a reversible TG2 inhibitor. The covalent inhibitors bind to the TG2 TGase site and lock TG2 in an open conformation which structurally disorders and inactivates the TG2 GTP binding site. The net effect is simultaneously inhibition of both the TGase and GTP binding activities.[6]

Ultraviolet light exposure is a major carcinogenic stimulus in epidermal cancer.[92] Consistent with a role of TG2 in skin inflammation, mouse epidermis releases the TNF-α, IL-6, and IL-8 inflammatory cytokines in response to ultraviolet light irradiation.[93] These responses are reduced in TG2 knockout epidermis.[93] Moreover, intracellular calcium level is increased in ultraviolet light treated keratinocytes, suggesting that calcium-dependent TG2 TGase activity mediates the inflammatory response.[93]

It is important to note that TG2 may not be the only transglutaminase that has a role in skin cancer, as the TG3 gene has been identified as a susceptibility locus for epidermal basal cell carcinoma.[94]

3.8 | Glioma/glioblastoma

High TG2 expression in patient tumors is associated with a poor prognosis.[95] Treatment with TG2 inhibitors, KC009 or ERW1227B, sensitives cultured glioblastoma cells to chemotherapeutic agents, and enhances apoptosis in glioblastoma xenografts,[96] and the GK921 TG2 inhibitor suppresses tumor cell transition to the mesenchymal phenotype.[95] In these models, inhibition of cell survival by the TG2 inhibitor, NC9, varies directly with TG2 content.[97] An interesting

finding is that some TG2 inhibitors, that are active in other cancer cell types,[6] are not highly active in glioblastoma cells.[97] One possible explanation is selective glioblastoma cell type-specific metabolism.[97] In another study, the NTU283 TG2 inhibitor was found to reduce TG2-dependent Akt/mTOR signaling in glioblastoma to suppress inflammatory responses.[98]

Glioblastoma includes the minimally aggressive neuronal and highly aggressive mesenchymal phenotypes. Highly aggressive mesenchymal glioblastoma cancer stem cells express elevated TG2.[99] Moreover, TG2 expression is dependent upon retinoic acid which is produced by aldehyde dehydrogenase 1A3 (ALDH1A3), suggesting that ALDH1A3, in glioblastoma stem cells, maintains TG2 level.[99] TG2 is a cancer stem cell maintenance factor,[18,84] but this finding suggests that a stem cell marker, ALDH1A3, can also induce TG2 level. C/EBPβ, TAZ, and STAT3 signaling also drives TG2 expression in mesenchymal phenotype cells.[95] This suggests that TG2 is a key protein driving this more aggressive phenotype.

Microvesicles, released from cancer cells, have been shown to transfer transforming information to adjacent cell types in tumors.[100] U87 glioblastoma cell studies show that TG2 and fibronectin are released in microvesicles which contain TG2-crosslinked fibronectin and that the TG2/fibronectin-positive vesicles activate mitogenic events in recipient cells[101] (Figure 5A). TG2 levels are elevated in the U87 and LN229 glioblastoma cell lines and this is associated with enhanced expression of EGFR and activation of EGFR signaling.[102] Moreover, forced overexpression of TG2 in glioblastoma increases EGFR expression and transforming activity by blocking c-Cbl-dependent EGFR ubiquitination.[102]

3.9 | Hepatocellular carcinoma

Proteomic analysis of recurrent hepatocellular carcinoma indicates a marked increase in TG2 level that this correlated with increased EMT marker expression.[103] Another study reports a substantial increase in TG2 in hepatocellular carcinoma and shows that inhibition of TG2 in HepG2.2.15 or Hep3B cell suppresses cell proliferation, invasion, and migration.[104] Moreover, cisplatin treatment of hepatocellular carcinoma cells reduces TG2 level which enhances cisplatin action.[105]

Acrylic retinoids inhibit post-surgery hepatocellular carcinoma recurrence via mechanisms that involve TG2 crosslinking of Sp1 transcription factor in the cell nucleus leading to loss of EGF receptor expression and cell apoptosis.[106] Moreover, this study shows that acrylic retinoids influence nuclear/cytoplasmic movement of TG2 in JHH-7 cells and that this is mediated via nuclear localization and export signals located on the TG2 protein.[107]

3.10 | Renal cell carcinoma

TG2 level is elevated in renal cell carcinoma[108,109] and elevated TG2 in primary tumors predicts enhanced metastasis and reduced disease-free survival.[17,110] It is interesting that the miR-1285 microRNA regulates TG2 in renal cancer cells. Reduced miR-1285 expression correlates with increased TG2 expression.[108] Moreover, silencing of TG2 or forced overexpression of miR-1285, reduce TG2 level and cell proliferation.[108] TG2 also regulates renal carcinoma cell survival, as TG2 knockdown leads to increased p53 level and enhanced apoptosis in 786-O, A498, CAKI-1, and ACHN cells.[109] TG2 crosslinking of p53 is the mechanism that leads to p53 loss and increased cell survival[109,111] (Figure 5B). This suggests that TG2 facilitates renal carcinoma cell survival by depleting p53. Moreover, treatment with GK921, a TG2 inhibitor, reduces ACHN and CAKI-1 renal carcinoma cell tumor growth in xenograft models.[112]

Glycolysis is enhanced in renal carcinoma cells[113] and high TG2 level increases glucose consumption and lactate production, while decreasing mitochondrial aconitase level. These effects are reversed by TG2 knockdown.[113] The ubiquitination system also influences TG2 function, as TG2 level is reduced following modification by CHIP

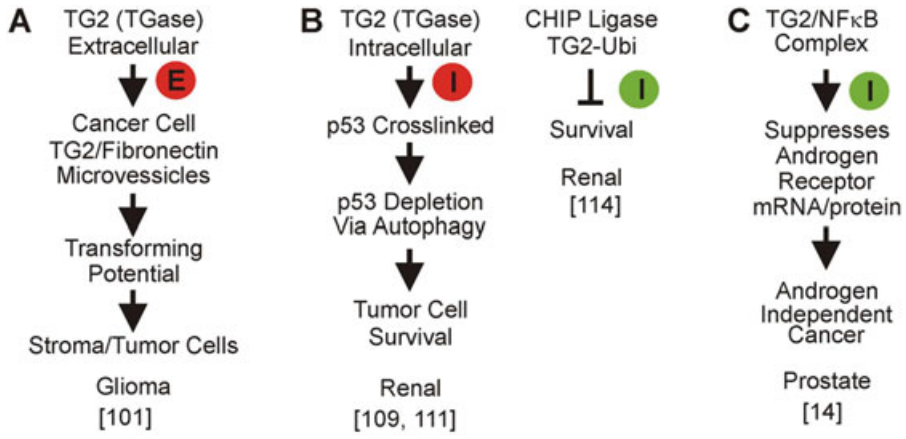

FIGURE 5 Transglutaminase signaling in glioma, renal and prostate cancer. The conventions are as indicated in Figure 1. A, The glioma reference is.[101] B, The renal cancer references are.[109,111,114] C, The prostate cancer reference is.[14] [Color figure can be viewed at wileyonlinelibrary.com]

(carboxyl-terminus of hsp70-interacting protein), an E3 ubiquitin ligase, and CHIP knockdown is associated with elevated TG2 and aggressive cancer[114] (Figure 5B). This may be a unique response in renal cancer cells, as we did not observe CHIP regulation of TG2 in squamous cell carcinoma (Eckert, unpublished).

TG2 also drives renal carcinoma cell adhesion and stemness. Silencing of TG2 in renal carcinoma cells reduces actin stress fiber formation and adhesion to fibronectin, collagen type I, and laminin,[115] leading to impaired invasion and migration, and reduced cancer stem cell marker expression.[115] These studies suggest that TG2 may by a viable cancer therapy target in renal cell carcinoma.[116]

3.11 | Prostate cancer

High level expression of TG2 in prostate cancer cells is associated with reduced androgen receptor expression via a mechanism that involves a reduction in androgen receptor mRNA and protein leading to attenuated response to androgen treatment[14] (Figure 5C). The reduction in androgen receptor expression is mediated via interaction of a TG2/NFκB complex with the androgen receptor gene promoter to reduce expression.[14]

TG4 may also have a role in prostate cancer. TG4 is uniquely expressed in normal prostate gland and elevated expression in human prostate tumors is associated with poor outcome and cancer recurrence.[117] TG4 has a role in prostate cancer cell survival, as it stimulates cell motility and N-cadherin expression, changes which are reversed by TG4 knockdown. These findings are consistent with a role for TG4 in maintaining EMT.[118] In addition, prostate cancer cells are more motile following forced TG4 expression and this involves TG2 co-localization and interaction with RON, the hepatocyte growth factor-like/macrophage-stimulating protein receptor.[119] In addition, TG4 protects prostate cancer cells from melanoma differentiation-associated gene-7 (MDA-7/IL-24)-dependent inhibition of cell adhesion, proliferation, and migration.[120] TG4 also co-precipitates and co-localizes with focal adhesion kinase and paxillin in prostate tumor cells and xenografts, and focal adhesion kinase inhibitors can negate the survival effects of TG4.[121] TG4 does not include a GTP/GDP binding domain, and it is interesting that this pro-cancer activity requires TG4 TGase activity.[121]

3.12 | Meningioma

Meningioma is a common central nervous system cancer that accounts for thirty percent of intracranial tumors.[122] Most are benign, but some form aggressive brain metastases. Microarray and immunostaining analysis reveal elevated TG2 in meningioma tumors which is directly correlated with high tumor grade, high labeling index and high incidence of post-surgery recurrence.[122] Consistent with a role of TG2 in meningioma cell survival, TG2 knockdown, or treatment with cystamine, induces meningioma cell death which is associated with reduced Akt signaling.[123] These studies suggest that TG2 may promote meningioma-related malignancy via an Akt signaling pathway.

3.13 | Mantle cell lymphoma

Mantle cell lymphoma (MCL) is an aggressive B-cell lymphoma that accounts for approximately 8% of non-Hodgkin lymphomas. TG2 is overexpressed in mantle cell lymphoma and is associated with poor prognosis.[124] TG2 is overexpressed in mantle cell lymphoma cells and activates NFκB signaling to increase STAT3 and IL-6 signaling which leads to enhanced autophagy-dependent cell survival.[125] In addition, autophagy has a positive feedback impact on IL-6 and TG2 signaling to further stimulate this survival mechanism.[126,127] TG2 knockdown leads to a reduction in autophagy and enhanced apoptosis.[126] Use of the proteasome inhibitor, bortezomib, is an approved therapy for mantle cell lymphoma. However, response to bortezomib therapy is modest and is associated with eventual chemoresistance and poor prognosis.[124] It is interesting that inhibition of TG2 TGase activity, using calcium blockers, increases interaction of p65 with IκBα to reduce NFκB signaling and enhance mantle cell lymphoma cell sensitivity to bortezomib.[125] How this relates to TG2 intracellular conformation is not known, but it may suggest that shifting TG2 to a closed conformation could reduce TG2 interaction with IκBα, thereby permitting NFκB/IκBα complex formation to reduce NFκB survival signaling.

3.14 | Melanoma

Melanoma is an aggressive skin cancer that is induced by exposure to ultraviolet irradiation.[128] Overexpression of TG2 in melanoma cancer cells promotes cell survival and drug resistance by enhancing integrin-mediated cell attachment.[129] This high level expression of TG2 can be exploited, as increasing TG2 TGase activity, by treatment with calcium ionophore, leads to apoptotic cell death[129] (Figure 6). TG2 has also been proposed as a ligand for GPR56 (adhesion G protein-coupled receptor). TG2 and GPR65 have antagonistic actions in melanoma cells.[130] TG2 TGase activity promotes melanoma cell growth, most likely by enhancing matrix deposition, while GPR56 binds to TG2 to inhibit this action and reduce fibronectin deposition[130] (Figure 6). The pro-cancer action of TG2 appears to require TG2 TGase activity, as expression of wild-type TG2, but not TGase inactive TG2, restores tumor formation in TG2 knockdown MC-1 cells.[130] In contrast, another study, using a unique model, suggests that TG2 is a melanoma metastasis suppressor. Tail vein injection of B16-F10 cells in C57BL/6N mice results in lung metastasis; however, tail-vein co-injection of B16-F10 cells with TG2 encoding plasmid increases lung TGase activity and reduces lung metastasis leading to prolong mouse survival.[131]

As expected, treating with stress/death inducing agents increases TG2 TGase activity to reduce melanoma cell survival. Aloe-emodin is a natural hydroxyanthraquinone that has anti-cancer properties. Aleo-emodin treatment of B16-F10 melanoma cells suppresses proliferation and invasion and enhances cell differentiation. This is associated with increased TG2 TGase activity leading to enhanced cell adhesion and aggregation[132] (Figure 6). In addition, strawberry fruit extract, which is rich in the anticancer anthocyanins, reduces B16-F10 melanoma cell proliferation and this is also associated with increased TG2 TGase

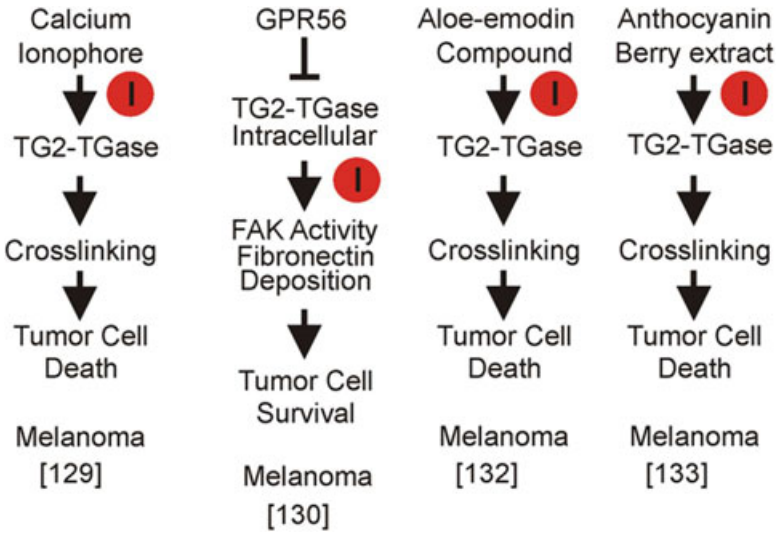

FIGURE 6 Transglutaminase signaling in melanoma. The conventions are as indicated in Figure 1. The references are.[129,130,132,133] [Color figure can be viewed at wileyonlinelibrary.com]

activity[133] (Figure 6). These findings suggest that TG2 TGase activity, and the associated crosslinking, is part of the cell death process.

3.15 | Esophageal cancer

TG2 level is elevated in advanced esophageal adenocarcinoma and this is associated with increased inflammation and a desmoplastic phenotype.[21] This increase is linked to increased TG2 gene copy number, and TG2 levels are further increased in chemotherapy and radiation-resistant cancer cells.[21] TG2 and NFκB levels are markedly increased in esophageal cancer, and TG2 expression is correlated with nodal metastasis and tumor invasion.[134]

TG3 has also been studied in esophageal cancer. TG3 levels are reduced in esophageal cancer cells and in patient tumor tissues[135] and this correlates with reduced patient survival.[136] Cell-based evidence shows that TG3 overexpression inhibits esophageal cancer cell proliferation, invasion, and migration and induces apoptosis via a mechanism that involves activation of NFκB signaling.[135] Thus, TG3 appears to suppress the transformed phenotype which is consistent with the known role of TG3 TGase activity in driving terminal cell differentiation.[45–47] However, cell signaling mediated via TG3 GTP binding, may also play a role, as TG3 encodes a GTP binding domain.[8,9]

3.16 | Osteosarcoma

TG2 function has also been studied in osteosarcoma, which is the most common solid-tumor bone cancer. Maintaining U2OS osteosarcoma cells in hypoxic conditions leads to a substantial increase in TG2 TGase activity.[137] In this context, TG2 protects cells from apoptosis by suppressing Bax level and reducing cytochrome c release, and these protective actions are attenuated by TG2 knockdown; however, it is not known if the TG2 TGase activity mediates the change in intracellular distribution of Bax and cytochrome c.[137] Elevated TG2 expression is also associated with osteosarcoma cell cisplatin resistance and TG2 knockdown restores chemosensitivity.[138] In addition, TG2 mRNA and protein level, and TGase activity, are elevated in MG-63 osteosarcoma lines maintained in hypoxic conditions.[139] This increase in TG2 is associated with elevated cytochrome c and caspase 3 expression and these responses are reversed by TG2 knockdown.[139]

3.17 | Cervical cancer

Cervical cancer is a major reproductive cancer in women. Increased TG2 and oncostatin M receptor levels are observed in clinical cervical cancer samples and correlate with disease progression.[140] TG2 appears to act with oncostatin M receptor to drive tumor cell invasion and migration, and TG2 knockdown attenuates these actions[140] (Figure 7A). In addition, TG2 interaction with α5/β1-integrin and fibronectin, and oncostatin M receptor enhances this interaction to maintain the malignant phenotype[140] (Figure 7A).

3.18 | Leukemia

Acute myeloid leukemia is associated with poor prognosis. Thus, there is an ongoing search for new therapy targets. Proteomic analysis shows that TG2 level is elevated in acute myeloid leukemia, and is further elevated in recurrent disease.[11] Moreover, elevated TG2 expression is associated with increased β3-integrin, fibronectin, FAK, and BclXL levels and reduced Bax level. Thus, TG2 is part of a protein network that predicts poor outcome.[11]

TG6 may also have a role in leukemia, as whole exome sequencing revealed a missense mutation in the TG6 gene which co-segregated with the cancer phenotype in a family, but was absent in healthy control patients.[141]

3.19 | Fibrosarcoma

Cardamonin, an anti-inflammatory flavonoid cancer prevention agent derived from plants, suppresses migration and invasion of HT-1080

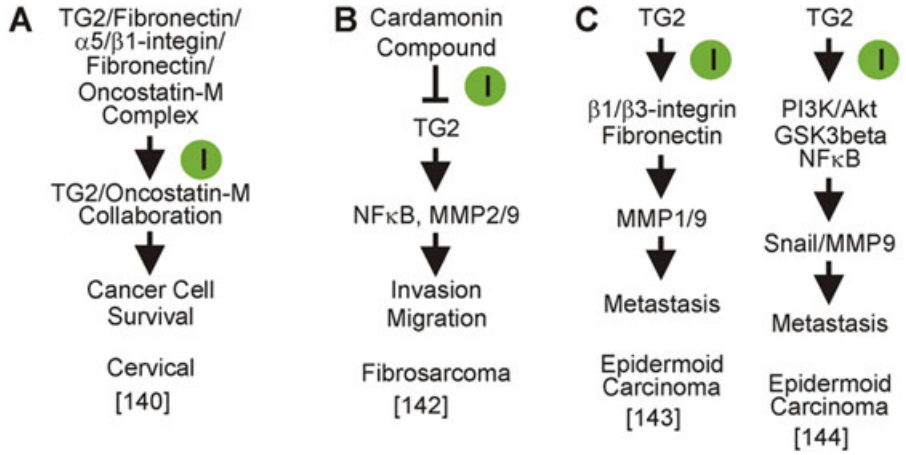

FIGURE 7 Transglutaminase signaling in cervical cancer fibrosarcoma and epidermoid carcinoma. The conventions are as indicted in Figure 1 and the flat head arrows indicate inhibition. A, The cervical cancer reference is.[140] B, The fibrosarcoma reference is.[142] C, The epidermoid carcinoma references are.[143,144] [Color figure can be viewed at wileyonlinelibrary.com]

sarcoma cells. As part of the mechanism of action, cardamonin suppresses TG2 level[142] (Figure 7B). Moreover, TG2 knockdown in HT-1080 cells suppresses invasion and migration, and this is associated with reduced NFκB, MMP-2, and MMP-9 level and activity. This study suggests that TG2 drives HT-1080 cell invasion and migration, and that cardamonin suppresses these events by reducing TG2 level[142] (Figure 7B).

3.20 | Epidermoid carcinoma

A431 epidermoid carcinoma cells express high levels of TG2.[143] Interestingly, knockdown of TG2 is associated with reduced β1-integrin interaction with fibronectin and reduced secretion of MMP-9 and MMP-1[143] (Figure 7C). Moreover, these changes are associated with reduced A431 cell attachment, migration, and invasion.[143] TG2 is also reported to induce PI3K/Akt activity and NFκB in A431 cells to increase Snail and MMP-9 expression leading to enhanced migration.[144] The increased NFκB activity is associated with TG2/NFκB complex formation, and the phenotype is reversed by PI3K, GSK3, or NFκB inhibitors[144] (Figure 7C).

3.21 | Laryngeal cancer

Analysis of laryngeal squamous cell carcinoma tumors isolated from 148 patients showed that high TG2 expression is associated with reduced survival in patients receiving postoperative radiotherapy, suggesting that TG2 has a role in cancer survival.[145]

TG3 level was also studied in this cancer and shown to be reduced in level in a study of seventy-two cases of laryngeal cancer.[146] TG3 is involved in assembly crosslinked structures during differentiation in stratifying epithelia[45–47] and so its loss is consistent with development of a less differentiated cancer-like phenotype.

3.22 | Mesothelioma

Mesothelioma is a rare cancer of the mesothelial cell layer of the pleura, peritoneum, pericardium and tunica vaginalis. Mesothelioma is caused by asbestos, is notoriously resistant to chemotherapy and is generally considered incurable with a poor life expectancy.[147] TG2 is increased in expression in mesothelioma cancer cells, maintained under hypoxic conditions, via a mechanism that involves HIF-1α[24] (Figure 8). TG2 knockdown or treatment with a TG2 inhibitor, in hypoxia-challenged cells, reduces cancer stem cell viability.[24]

Moreover, TG2 is highly expressed in mesothelioma cancer stem cells and knockdown results in reduced spheroid formation, matrigel invasion, migration, and tumor formation[148] (Figure 8). In xenograft mesothelioma tumor models, time to tumor first appearance is doubled from 9 weeks for wild-type cells, to 18 weeks for TG2 knockout cells. Moreover, TG2 loss is associated with reduced tumor expression of stemness and epithelial mesenchymal transition markers, and enhanced apoptosis[148] (Figure 8). Treatment with NC9, a TG2 inhibitor, markedly suppresses mesothelioma cell tumor growth, suggesting TG2 is a candidate mesothelioma therapy target.[148]

3.23 | Colon cancer

Recent findings suggest that TG2 is overexpressed in colon cancer,[149] and is a prognostic factor that predicts early recurrence.[26,150] Moreover, TG2 level is markedly elevated in cultured human colon cancer stem cells, and knockdown reduces cell proliferation, invasion, EMT, and stem cell marker (CD133, SOX2, and β-catenin) expression.[151] Likewise, TG2 expression in RKO, SW480, or SW620 colon cancer cells correlates with increased expression of stem cell (CD44) and EMT markers and xenograft tumor formation.[152] TG2 and TGFβ1 appear to function in a positive autocrine feedback loop,[152] and TG2 expression leads to increased nuclear β-catenin accumulation in SW620 cells.[152] These findings suggest a role for TG2 in driving cancer stem cell maintenance in colon cancer.

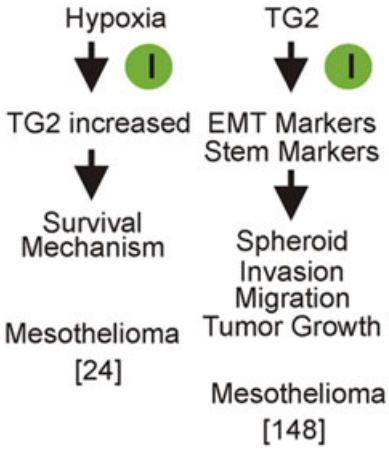

FIGURE 8 Transglutaminase signaling in mesothelioma. The conventions are as indicted in Figure 1. The mesothelioma references are.[24,148] [Color figure can be viewed at wileyonlinelibrary.com]

However, other studies suggest that TG2 is inversely correlated with aggressive colon cancer cell behavior. TG2 knockdown in SW480 cells, a modestly aggressive colon cancer cell type, increases invasive behavior in a matrigel invasion assay. Moreover, TG2 overexpression in SW620 cells, a highly aggressive cell line, reduces invasive potential.[153] In addition, miR-19, a microRNA that inhibits TG2 level, is increased in malignant SW620 cells that express reduced TG2 levels.[153] Given that cells transfected with wild-type TG2, but not with the TGase inactive TG2, display reduced invasive behavior, the authors conclude that TG2 inhibits invasive behavior in a cross-linking dependent manner.[153] In addition, a comparison of colon cancer cell lines indicates an inverse correlation between an aggressive phenotype and TG2 TGase activity.[154] A study using CT26 mouse colon cancer cells shows smaller tumor formation when engineered to overexpress wild-type TG2, but not a TGase activity-inactive mutant. This was attributed to TG2 stimulation of TGFβ1 production and assembly of extracellular matrix, leading to increased cancer cell adhesion and reduced migratory and invasive behavior.[155]

4 | TG2 EXPRESSION IS ASSOCIATED WITH AGGRESSIVE HUMAN DISEASE

4.1 | TG2 tumor expression and association with disease progression

Studies of human tumor samples reveal that TG2 level is elevated in gastric, oral, breast, ovarian, lung, pancreatic, renal, esophageal, mesothelioma, cervical, lymphoma, meningioma, leukemia, and colon cancer, and correlates with advanced tumor stage, increased metastasis, poor prognosis, and disease recurrence (Table 1). These findings are consistent with pre-clinical studies showing that TG2 enhances cancer cell survival and proliferation, migration, and matrigel invasion, suggesting a pro-cancer role. However, the mechanism of TG2 action varies. For example, GTP-bound TG2 drives gastric, breast, epidermal squamous cell carcinoma and lung cell survival, while in ovarian and pancreatic cancer, extracellular TG2 TGase activity modifies matrix to enhance cancer cell adhesion to increase survival (Table 1). In glioblastoma TG2 is bundled into vesicles with fibronectin which are then released to enhance tumor cell survival.[101] In renal cell carcinoma, cancer cell survival is increased due to TGase modification and inactivation of p53.[109,111] Thus, both GTP-bound/signaling-active and calcium-bound/transamidase-active TG2 conformations have been reported to enhance cancer cell survival.

In addition, TG2 TGase activity has a role in cell death, which is induced by treatment with exogenous pro-apoptotic agents or calcium ionophore. For example, treatment of melanoma cells with aloe-emodin[132] or anthocyanins[133] induces TGase-associated cell death. Among these studies, the colon cancer findings are the most puzzling. TG2 levels are elevated in human colon tumor tissue and this is associated with aggressive disease[26,149,150]; however, preclinical studies suggest that TG2 has survival and anti-survival roles.[151–155]

5 | ROLE OF TG2 GTP/GDP BINDING VERSUS TGASE ACTIVITY

It is essential that we determine if the GTP-bound/closed/folded/signaling-active, calcium-bound/open/extended/transamidase-active, or both TG2 forms drive cancer cell survival and tumor progression in each tumor type. Identifying the role of these two conformations has been studied using two approaches. The first involves expression of TG2 GTP binding-deficient or TGase activity-deficient mutants in cells and assessing the impact on the cancer cell phenotype. This approach has been used in breast,[30,53] ovarian,[69] lung,[78] epidermal squamous cell carcinoma,[18] and colon cancer.[153] The second method is direct observation of intracellular TG2 conformation using FRET probe-labeled TG2 constructs and FRET-FLIM microscopy. This approach has been used to study TG2 conformation in immortalized normal mouse cells,[7] lung cancer cells,[156] and epidermal squamous cell carcinoma cells.[6] Studies in immortalized normal mouse cells showed that TG2 shifts to the open conformation following treatment with TG2 inhibitor, NC9,[7] suggesting that this TGase site-interacting covalent inhibitor locks TG2 in an open/extended conformation. The A549 lung adenocarcinoma cell study suggests that TG2 assumes a closed/folded conformation when localized in perinuclear endosomes and an open/extended conformation when localized near the plasma membrane.[156] The TG2 FRET study in epidermal squamous cell carcinoma cells showed that TG2 inhibitors, including NC9, VA4, and VA5, lock TG2 into the open/extended conformation[6] and further showed that this distorts the TG2 GTP binding site to block TG2 GTP/GDP binding activity. Thus, inhibitors like NC9 block both TG2 GTP binding and TGase activities.[6,7] Because both the GTP-bound and TGase-active TG2 conformations can drive cancer development, in a tumor-specific manner, it is fortuitous that these inhibitors suppress both activities.

Considering this information, we propose the scheme in Figure 9 as a general model of how TG2 may regulate cancer cell life and death.

TABLE 1 TG2 action at a glance[a]

Cancer type	Signal transduction	GTP/GDP binding, TGase activity	Clinical relevance
Gastric cancer	ERK1/2[25] NFκB/HIF-1α[36]	GTP binding (intracellular, survival[25,36])	TG2 elevated in tumors correlated with tumor stage[25]
Oral squamous cell carcinoma			TG2 elevated in tumors and metastasis, associated with poor prognosis[22]
Breast cancer	EGFR, Ras, Cdc42, PI3K, NFκB,[52] Snail, Zeb, Zeb2, Twist,[58,60] TGFβ,[58] Stem cell markers,[58,59] NFκB,[30,53] Akt, NFκB,[63] S100A4[65]	GTP binding (intracellular, survival),[30,53,60] TGase[62]	TG2 elevation in stroma predicts cancer recurrence[48,49]
Ovarian cancer	Src, β-catenin [70], TGFβ, fibronectin [71,72], Wnt/β-catenin [72,74] and IL-6 [73]	TGase (extracellular crosslinking, survival)[69]	TG2 elevated in tumors[69]
Lung cancer	p53,[15,77] TGFβ1, JNK, PP2A[79]	GTP binding (intracellular, survival)[78]	TG2 elevated in tumor predict disease recurrence, prognostic marker[75,76]
Pancreatic cancer	YAP1/TAZ,[83] eEF2 kinase,[86] Snail, ZEB1[86]	TGase (extracellular crosslinking, survival)[83]	TG2 elevated in tumors, associated with reduced survival[82]
Epidermal squamous cell carcinoma	α6/β4-integrin, FAK, Src, PI3K, PDK1, YAP1, ΔNp63α,[18,84,88] VEGFA, NRP1,[89,90] TNF-α, IL-6, IL-8[93]	GTP binding (intracellular, survival)[18,84]	
Glioma/glioblastoma	Akt, mTOR,[98] C/EBPβ, TAZ, STAT3,[95] EGFR, c-Cbl[102]	TGase (extracellular vesicles, signal transfer, survival)[101]	
Hepatocellular carcinoma	Sp1, EGFR[106]	TGase (retinoid activation of TGase, apoptosis)[106]	
Renal cell carcinoma	p53[109,111], mitochondrial aconitase[113]	TGase (intracellular p53 crosslinking and inactivation, survival)[109,111]	TG2 elevated in tumors, predicts metastasis[17,110]
Prostate cancer	Androgen receptor, NFκB[14]		
Meningioma	Akt[123]		TG2 is elevated in aggressive disease and predicts recurrence[122]
Mantle cell lymphoma	NFκB, STAT3, IL-6[125]		TG2 elevated in tumors, predicts poor prognosis[124]
Melanoma	adhesion G protein-coupled receptor[130]	TGase, (intracellular activation with ionophore or anthocyanins, cell death)[129,133]	
Esophageal cancer	NFκB[134]		TG2 elevated in tumors and associated with inflammation, nodal metastasis, and tumor invasion[21,134]
Osteosarcoma	Bax, cytochrome c, caspase 3[137,139]		
Cervical cancer	Oncostatin M receptor, α5/β1 integrin, fibronectin[140]		TG2 elevated in tumors and correlates with disease progression[140]
Leukemia	β3-integrin, fibronectin, FAK, BclXL, Bax[11]		TG2 elevated in tumors, predicts recurrent disease[11]
Fibrosarcoma	NFκB, MMP-1, MMP-9[142]		
Epidermoid carcinoma	β1/β3-integrins, fibronectin, MMP-1, MMP-9,[143] PI3K, GSK3, NFκB[144]		
Laryngeal cancer			TG2 elevated in tumors, predicts reduce patient survival[145]
Mesothelioma	HIF-1α,[24] stemness, EMT, apoptosis markers[148]		TG2 increased in tumors[148]
Colon cancer	CD133, SOX2, β-catenin,[151,152] CD44, EMT markers,[152] TGFβ1[155]	TGase (intracellular activity, survival)[153,155]	TG2 elevated in tumors, associated with early recurrence[26,149,150]

Signal transduction = pathways/endpoints modulated by TG2 in cells and/or tumors. Clinical Relevance = impact of TG2 level on disease outcome. GTP/GDP binding versus TGase activity = TG2 activity involved in indicated response (cell survival or death).

[a]See text for additional explanation of information in table.

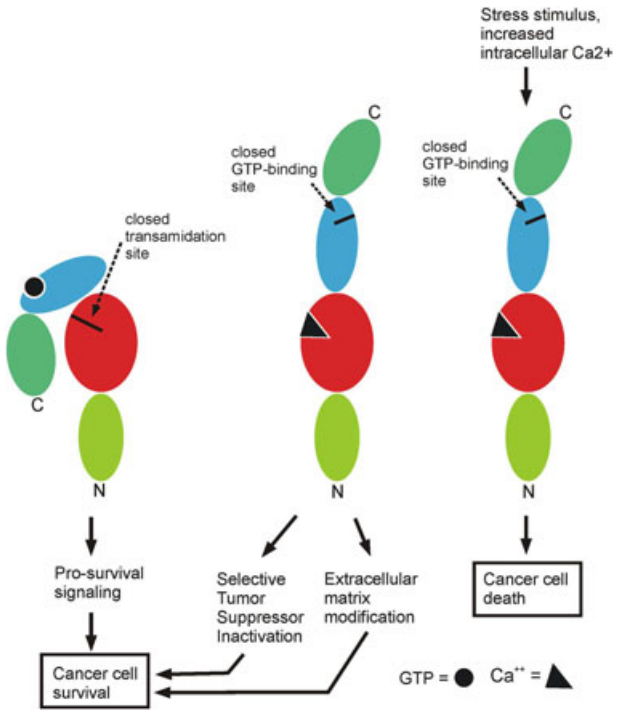

FIGURE 9 Transglutaminase 2 and cancer cell survival and death. The closed/folded/signaling-active GTP-bound conformation of TG2 (left model) drives intracellular cancer cell, and cancer stem cell survival signaling. The open/extended transamidase-active conformation of TG2 (middle model) can enhance cancer cell survival by selective intracellular tumor suppressor inactivation or after release to the extracellular environment can modify the extracellular matrix to enhance cancer cell survival. The open/extended transamidase-active conformation of TG2 (right model) can enhance cancer cell death when activated by an intracellular stimulus that enhances intracellular calcium level. The black circle represents GTP binding to the TG2 GTP/GDP binding domain. The black triangle represents calcium binding to the TGase site to active transamidation and protein crosslinking [Color figure can be viewed at wileyonlinelibrary.com]

Evidence suggests that the GTP-bound/closed/folded/signaling-active conformation stimulates downstream pro-survival signaling pathways to enhance cancer cell survival. Other studies suggest that the open/extended transamidase-active conformation (calcium bound) can selectively transamidate key tumor suppressor proteins or modify extracellular matrix, to enhance cancer cell survival. In addition, high level expression of TG2 or treating cells with stress agents, that compromise plasma and endoplasmic reticulum membranes to increase intracellular calcium, cause massive activation of the open/extended transamidase-active form leading to crosslinking activation and cell death.

6 | SUMMARY—TG2 AND CANCER CELL LIFE AND DEATH

These studies support several important conclusions. First, TG2 levels are elevated in all cancers where it has been examined, and that elevated expression is associated with poor prognosis, disease recurrence, advanced disease, metastasis, poor prognosis, and reduced patient survival. Second, TG2 conformation is a key determiner of pro-cancer activity. The GTP bound form of TG2, which predominates in the intracellular environment, is a major driver of cancer cell survival signaling, but the calcium-bound transamidase form, which is present in the extracellular and intracellular environments, can also drive disease progression. The GTP bound form appears to be the predominant driver of cancer (see green dots, Figures 2-8). Third, treatment with extracellular agents, that increase intracellular calcium level, can kill cancer cells via activation of TG2 transamidase activity.[132,133] Fourth, TG2 inhibitors, currently in development, which inhibit both TG2 GTP binding and transamidase activity, have shown efficacy without toxicity in pre-clinical studies[6,28,41,42,84,88] and hold great promise as anti-cancer therapeutic agents.

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