

REVIEW ARTICLE

Transglutaminase 2: A keystone in the molecular puzzle of cancer

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Abstract

Transglutaminase-2 (TG2), an enzyme also referred to as tissue transglutaminase, is ubiquitously present in a wide range of tissues, cell types, and subcellular compartments. Extensive research has demonstrated its involvement in both physiological cellular processes and pathological conditions. Studies investigating the diverse range of actions and multitude of targets associated with TG2 have significantly contributed to the understanding of its role in various types of cancer. This association is established through its interaction with pathways implicated in the initiation, progression, and ultimate spread of tumors. Furthermore, recent findings indicate that TG2 has a role in modifying the biomechanical milieu and signaling inside the tumor microenvironment, alongside mediating cancer cell behavior and intracellular signaling. In this review, we aim to elucidate the existing understanding of TG2's involvement in cancer, with a specific emphasis on its functions in translating external signals into the initiation of oncogenic processes. Enhanced comprehension of these pathways may potentially pave the way for novel treatment approaches aimed at modulating this versatile protein.

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1. Introduction

Transglutaminase-2 (TG2), an enzyme also known as tissue transglutaminase, is ubiquitously present in a wide range of tissues, cellular types, and subcellular compartments. Extensive research has demonstrated its involvement in both physiological cellular functions and pathological conditions.¹ The transglutaminase enzyme family exhibits significant expression, with one member, in particular, being notably prevalent. This enzyme primarily functions by catalyzing the synthesis of lysine-glutamine isopeptide bonds in a calcium ion (Ca²⁺)-dependent manner, resulting in the cross-linking of proteins by transamidation. In addition to its known enzymatic activities, TG2 has been associated with additional functions such as deamidation and GTPase signaling. Extensive research has explored each of these enzymatic functions within the field of cancer biology. The TG2 protein comprises four discrete globular domains, including an N-terminal β -sandwich region that encompasses a site responsible for binding to fibronectin and integrin. In addition, there is a domain

housing a catalytic triad composed of Cys277, His335, and Asp358, which primarily facilitates acyl-transfer reactions. This domain also contains a conserved tryptophan residue. Furthermore, there are two β -barrel domains, one of which contains a sequence that binds to phospholipase C, while the other encompasses the protein's C-terminus.²

Over the past 15 years, numerous studies have demonstrated a correlation between TG2 and cancer progression. Increased expression of the protein has been observed in glioblastoma,³ ovarian,⁴ pancreatic,⁵ lung,⁶ and breast cancer.⁷ In addition, a link between poor clinical outcomes and pancreatic, ovarian, and lung cancer has been observed, supporting the notion of TG2 functioning as a tumor promoter. TG2 can activate various oncogenic pathways associated with cancer progression, including nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), focal adhesion kinase (FAK), protein kinase B (Akt), β -catenin, ras homolog family member A (RhoA), and yes-associated protein 1 (YAP).⁸

Three overarching themes have been identified. First, TG2 promotes chemotherapy and radiation resistance by activating the NF- κ B survival pathway or by facilitating “outside-in” communication that begins with TG2-regulated cellular adherence to the matrix.⁹ Second, TG2 has been associated with metastasis; in ovarian orthotopic xenograft models, peritoneal dissemination was reduced when TG2 was knocked down in ovarian cancer cells.⁴ Discoveries revealed that TG2 controls cancer cell adherence to the matrix and induces epithelial-mesenchymal transition (EMT). These early findings in ovarian cancer models were confirmed in models for lung and breast cancer. Last but not least, TG2 has been demonstrated to be strongly expressed in cancer stem cell models of ovarian, cutaneous, breast, and brain cancers. In addition to its roles inside cancer cells, TG2 is also expressed in stromal cells, exerting an impact on tumor growth, as elucidated in the present review.

2. Hallmarks of cancer and TG2

Cancer exhibits significant heterogeneity at the genetic and molecular level, characterized by numerous subtypes, mutational backgrounds, and organs of origin. Despite this wide variability, the overall concept of normal cells progressing to a cancerous state has been effectively conceptualized as a step-by-step acquisition of six fundamental hallmarks of cancer.¹⁰ The primary characteristics include maintaining the ability to continuously signal for cell development, avoiding mechanisms that restrict cell growth, resisting cell death, allowing cells to replicate indefinitely, promoting the formation of new blood vessels, and initiating the invasion

of surrounding tissues. TG2 is involved in several processes associated with these characteristics, such as EMT, survival of cancer stem cells, resistance to drugs, signaling related to inflammation and proliferation, as well as invasive and metastatic behavior¹¹ (Table 1).

Other signaling pathways also connect TG2 to cancer progression. A study has demonstrated that downregulation of TG2 inhibits glioma stem cell growth by lowering the expression of inhibitor of DNA binding 1 (ID1) protein.¹⁹ On the other hand, overexpressing ID1 restores the proliferation of the cells, demonstrating that ID1 activates the phosphoinositide 3-kinase (PI3K)/AKT pathway and acts as a downstream mediator of TG2. In addition, TG2 has been linked to the buildup of β -catenin, which is often triggered by the Wnt pathway.⁸ β -catenin then translocates to the nucleus, where it increases the production of cyclin D1 and c-Myc, ultimately promoting the proliferation of ovarian cancer cells. TG2 may control the Wnt pathway by directly interacting with β -catenin. Its nuclear interactome consists of several proteins that are known to participate in regulating the Wnt signaling.¹³ Apart from that, TG2 has been demonstrated to stimulate the growth of gastric cancer cells through the ERK1/2 pathway.¹² Cellular proliferation was reduced with TG2 knockdown, and a partial reversal of proliferation was observed upon the addition of a particular ERK1/2 inhibitor, indicating that the ERK1/2 pathway may play a mediating role in TG2-driven proliferation.

The activity of TG2 has been recognized to regulate the function of tumor suppressors. Insufficient phosphorylation of the retinoblastoma protein (RB) prevents cells from dividing by altering the activity of transcription factors that

Table 1. Transglutaminase-2 and the hallmarks of cancer

Hallmark	Key pathways	References
Sustaining proliferative signals	B-catenin/Wnt, TGF- β , ERK1/2, PI3K/AKT	12,13
Resisting cell death	TRAIL, Caspase-3/Bax	14
Evading growth suppressors	RB/p53 pathway regulation	15
Inducing angiogenesis	NF- κ B/HIF1 α , VEGF, ECM remodeling	16
Enabling replicative immortality	EMT, CSCs (CD44), YAP/TAZ	17
Activating invasion	EGF, EMT/TGF- β , Rac, ECM alterations	18

Abbreviations: RB: Retinoblastoma protein; TGF- β : Transforming growth factor beta; PI3K: Phosphoinositide 3-kinase; TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; NF- κ B: Nuclear factor kappa B; HIF1 α : Hypoxia-inducible factor1 α ; VEGF: Vascular endothelial growth factor; ECM: Extracellular matrix; EMT: Epithelial-mesenchymal transition; EGF: Endothelial growth factor.

control the expression of genes involved in transitioning from the G1 to S phase of the cell cycle.²⁰ Multiple studies have shown that RB serves as a substrate for TG2 kinase activity,²¹ facilitating an anti-apoptotic impact through RB phosphorylation. Previous research on TG2 demonstrated that RB acted as a substrate in lymphoma cells during apoptosis. In addition, TG2 was found to safeguard RB against degradation caused by caspases through a transamidation-dependent mechanism.²²

Undoubtedly, cancer is complex and involves a network of signals and interactions that contribute to its development. TG2 plays a crucial role in enhancing cancer characteristics, and its function must be understood within the broader network. TG2 helps maintain proliferative signals through the β -catenin/Wnt and ERK1/2 pathways, while also interacting with the Hedgehog and Notch pathways, which are known to play important roles in cell proliferation.²³ These mechanisms either enhance or modulate the impact of TG2 on cell proliferation. In clinically aggressive meningioma, there is an elevated expression of TG2, correlating with higher World Health Organization malignancy grading and recurrence rates. Blocking TG2 with small interfering RNA (siRNA) or cystamine leads to meningioma cell death and inhibits cell growth by reducing AKT phosphorylation and activating caspase-3.²⁴ The Sp1 transcription factor inhibits TG2 expression in neuroblastoma by binding to the respective promoter.²⁵ TG2 levels are significantly elevated in metastatic patients with primary renal cell carcinoma compared to non-metastatic individuals, suggesting its link with cancer spread and metastasis.

3. Role of TG2 in tumor progression

The relationship between TG2 and cancers has long been a subject of intense discussion and ongoing study. TG2 is typically expressed and activated most prominently in the bladder, liver, and adrenal glands. In the majority of other normal tissues, TG2 is less common and often restricted to the epithelium or nearby stroma. Functioning as a protein-crosslinking enzyme, TG2 is widely expressed and dependent on Ca^{2+} . Moreover, it acts as a G-protein with dual capabilities.²⁶ The gene responsible for encoding TG2 is regulated by a stress-responsive mechanism and serves as a transcriptional target of hypoxia-inducible factor (HIF)-1 α . In addition, TG2 forms a bond with fibronectin and possesses transamination functionality. TG2 plays crucial roles in regulating cell survival, differentiation, proliferation, migration, invasion, and apoptosis, as well as in influencing cellular interactions with the microenvironment. The persistent expression of TG2 in cancer cells results in the continuous activation of FAK and the subsequent activation of the PI3K/Akt survival pathway.²⁶

The involvement of TG2 in tumor biology extends beyond its enzymatic and signaling roles, directly influencing the cellular cytoskeleton. The enzyme plays a crucial role in catalyzing the cross-linking of cytoskeleton proteins inside cells in the presence of calcium, which holds significant implications for cancer progression.¹ This action can stabilize the cytoskeleton, thereby hindering the growth of tumor cells by altering their structural integrity and mechanical characteristics. Such findings suggest that TG2 may exhibit a tumor-suppressive function in certain circumstances, underscoring the intricate nature of its role in cancer biology. The ability of TG2 to polymerize the cytoskeleton, which is dependent on calcium levels, highlights its multifaceted impact on the tumor microenvironment (TME). This impact extends beyond cell survival and proliferation to encompass mechanical dynamics essential for tumor cell motility and invasiveness. However, high levels of intracellular polyamines in tumors notably affect the activity of TG2, contrasting its possible anti-tumor action.²⁷ Polyamines can impede the transamidation function of TG2, thereby preventing the enzyme from providing stability to the cytoskeleton. This inhibition fosters a more invasive and aggressive tumor phenotype by promoting cellular pathways that support migration and invasion. The enzymatic activity of TG2, influenced by calcium and polyamines, demonstrates the delicate balance between pro-tumorigenic and anti-tumorigenic factors in cancer cells.

TG2 may hinder the progression of apoptosis and contribute to cellular growth. While TG2 is often downregulated in initial tumors and during tumor development, it is commonly upregulated in secondary malignancies and those that are resistant to chemotherapy.²⁸ The involvement of TG2 in tumor development is attributed to its regulation of malignancy mobility and invasion. In addition, the expression of TG2 is associated with the activation of pathways that have established significance in the progression of cancer.²⁹ Cellular motility on fibronectin is markedly reduced with TG2 inactivation. Fibronectin-bound TG2, irrespective of its activity, may enhance integrin-independent cellular adhesion. In collagen- or Matrigel-coated transwell plate assays, cancer cells harboring TG2 exhibit enhanced cell motility and invasion capabilities.³⁰

The extracellular matrix (ECM) regulates angiogenesis and the spread of malignant cells significantly. TG2 may impact ECM and intracellular adhesion in several ways. By hydrolyzing TG2 on the cellular surface at the interface between malignant and normal tissues, matrix metalloproteinase 1 (MMP1) and MMP2 may inhibit fibronectin-mediated cancer cell adhesion and

migration. However, this process can enhance collagen-mediated tumor cell migration.³¹ In addition to inhibiting angiogenesis, exogenous TG2 may reduce MMP-induced ECM breakdown. TG2 enhances cancer cells' capacity to invade by influencing the ECM. It has the potential to crosslink numerous ECM proteins, thereby increasing fibronectin stability during the early stages of matrix formation. Consequently, the adhesive power and extensibility of fibroblasts that overexpress TG2 are considerably enhanced.³² The integrity of the ECM relies on TG2-dependent cross-linking, and this TG2 activity creates a barrier against tumor spread. *In vivo*, active TG2 may induce the accumulation of a complex ECM, which might inhibit the formation of endothelial tubes without resulting in cell death. The ECM may become highly robust and resistant to proteolytic degradation with the help of TG2. TG2 is highly expressed in the stroma of tumors with negative lymph nodes, and it is catalytically active.²⁶ Robust suppression of cancer cell invasion through the Matrigel transwell filters is also obtained by pretreating Matrigel with catalytically active TG2. Therefore, TG2-induced changes in the ECM may serve as an efficient inhibitor of metastasis, and a possible strategy to prevent metastasis may include selectively introducing catalytically active TG2 to the tumor location.³³ The role of TG2 as an anti-apoptotic or pro-apoptotic protein is dependent on the specific cell type and its subcellular distribution. An elevated concentration (>1 mM) of Ca²⁺ triggers the activation of TG2, facilitating the formation of inter- and intra-molecular crosslinks among proteins while leading to cellular death.²⁶ The use of TG2-specific antisense RNA has the potential to provide cellular protection against stress-induced cell death. On the contrary, cellular conditions characterized by a low concentration of Ca²⁺ (<1 mM) and a high concentration of GTP (>9 mM) often facilitate the survival of cell signaling mediated by TG2.³⁴ Moreover, TG2 influences tumor growth and metastasis by regulating many biomechanical and biological processes inside the TME (Figure 1).

4. Essential role of TG2 in TME

4.1. TG2 and the immune system in cancer

The interplay among inflammation, the immune system, and cancer is intricately linked to its development and ultimate outcome.³⁵ Despite the identification of TG2 in inflammatory signaling across various conditions, including sepsis,³⁶ celiac disease,³⁷ cystic fibrosis,³⁸ and fibrosis,³⁹ a potential triangular association involving the immune system, tumor, and TG2 has yet to be extensively investigated. The association between TG2 and transforming growth factor beta (TGF- β) generates a robust pro-inflammatory feedback loop, which is also

present in the relationship between TG2 and NF- κ B, the primary transducer of inflammatory signaling.¹ By polymerizing the inhibitory NF- κ B inhibitor alpha (I κ B α) subunit or inducing phosphorylation of RelA/p65, TG2 mediates NF- κ B-mediated signaling.⁴⁰ Furthermore, TG2 is reciprocally controlled by an NF- κ B binding motif in its promoter domain.⁴¹

The expression of TG2 has been shown to be a determining factor in the production of the pro-inflammatory cytokine interleukin (IL)-6 within the TME.⁴² The production of IL-6 has been linked to the development of a stem cell phenotype, as well as its role in the process of EMT.⁴³ The upregulation of IL-6 expression was shown to be reliant on TG2, further amplified through signaling cascades involving NF- κ B, PI3K, and JNK. This upregulation of IL-6 played a crucial role in supporting a cancer stem cell phenotype and triggering the process of EMT and metastasis. In addition, the synthesis of IL-1 β was found to enhance the amplification of IL-6 expression.⁴⁴

In the TME, several types of cancer are accompanied by a notable presence of immune cells. However, malignant cells possess the ability to evade and suppress this immune response, even when confronted with substantial inflammatory signaling.⁴⁵ The crucial involvement of macrophages in the TME is evident, with tumor-associated macrophages (TAMs) representing the predominant immune cells strongly connected with the disease progression.⁴⁶ Studies on central nervous system inflammation have provided data indicating that TG2 has the ability to facilitate the recruitment of macrophages,⁴⁷ a phenomenon observed as an initial occurrence in several cancer types. Macrophages can differentiate into two distinct lineages known as M1 and M2. The M1 lineage is characterized by its involvement in pro-inflammatory processes, whereas the M2 lineage is associated with immunological resolution.⁴⁸ While research on the relationship between TAMs, ECM remodeling in cancer,⁴⁹ and TG2 is lacking, mounting evidence suggests that TAM/cancer-associated fibroblasts-mediated remodeling is essential for evading anti-cancer immune responses.⁵⁰ Therefore, further studies in this area would be worthwhile.

It is worth mentioning that DNA interaction through the STING pathway initiates specific immune effector responses that may influence various stages of carcinogenesis, from cancer cell transformation to metastasis.⁵¹ Upon recognizing microbial DNA, the STING pathway triggers the production of type I interferons and inflammatory cytokines, thereby promoting anti-microbial innate immunity. Recent research has shown that the cGAS-STING pathway may be activated by self-DNA from tumors and by-products of genomic instability, which can

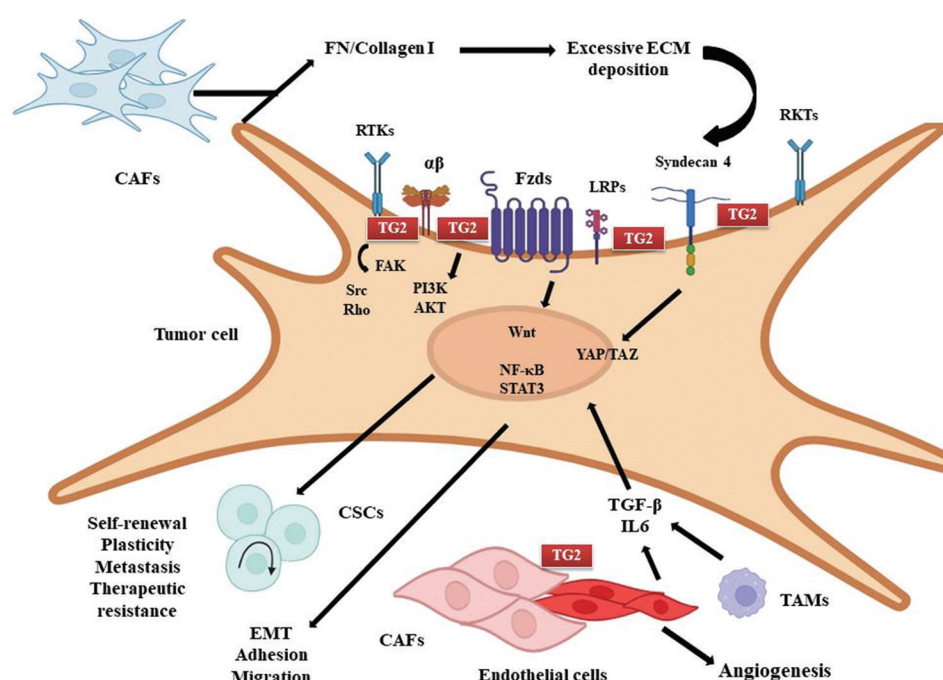


Figure 1. Role of TG2 in cancer. A variety of cellular populations within the TME, such as tumor cells, CAFs, infiltrating immune cells, and endothelial cells, release ECM macromolecules (FN and collagen I) and cytokines (TGF- β and IL-6). TG2 is released into the ECM region at the cellular level, where it interacts with FN, collagen I, TGF- β , and other growth factor receptors, integrin β subunits, Wnt receptors, and syndecan-4. These connections initiate intracellular signaling pathways, including FAK, RhoA, and PI3K/Akt, as well as oncogenic signaling (Wnt, YAP/TAZ, NF- κ B), which play a role in cancer development, progression, angiogenesis, stem cell maintenance, and resistance to treatment.

Abbreviations: AKT: Protein kinase B; CAFs: Cancer-associated fibroblasts; CSCs: Cancer stem cells; ECM: Extracellular matrix; EMT: Epithelial-mesenchymal transition; FAK: Focal adhesion kinase; Fzds: Frizzled proteins; FN: Fibronectin; IL6: Interleukin 6; LRP: Leucine-responsive regulatory proteins; NF- κ B: Nuclear factor kappa B; PI3K: Phosphoinositide 3-kinases; RKTs: Receptor tyrosine kinases; STAT3: Signal transducer and activator of transcription 3; TAMs: Tumor-associated macrophages; TAZ: Transcriptional co-activator with PDZ-binding motif; TG2: Transglutaminase-2; TGF- β : Transforming growth factor-beta; TME: Tumor microenvironment; Wnt: Wingless-related integration site; YAP: Yes-associated protein.

either enhance or hinder tumor growth. Consequently, cancer treatments utilizing STING agonists, both alone and in combination with traditional cancer treatments or immune checkpoint targeting, have been developed.⁵² Furthermore, studies have demonstrated that TG2 inhibits IRF3 phosphorylation in macrophages derived from bone marrow, resulting in the negative regulation of STING signaling.

The enzymatic activities of TG2 within the TME are crucial for regulating the ECM and cellular adhesion, creating an intricate interaction that may either promote or hinder tumor progression.⁵³ The capacity of TG2 to cross-link ECM proteins, such as fibronectin, strengthens the stability and integrity of the ECM, which may limit tumor cell migration and infiltration.²⁶ Moreover, TG2 activity leads to the creation of a physical barrier that might hinder the dissemination of cancer cells, suggesting its possible function in tumor suppression. Understanding the conflicting functions of TG2 in the TME is essential because its actions might have opposing impacts on

cancer progression. The necessity of recognizing the cellular environment is highlighted by the delicate balance between the ECM-stabilizing effects of TG2 and its ability to promote tumor invasiveness.⁵⁴

4.2. TG2 and angiogenesis induction

The process of cancer-associated angiogenesis involves the continuous activation of the angiogenic switch, inducing the previously inactive blood vessels transition into sustained angiogenesis, thereby supporting tumor growth.⁵⁵ Hypoxia triggers the secretion of vascular growth factors, such as vascular endothelial growth factor (VEGF), promoting angiogenesis. In addition, resident vascular cells, including pericytes and endothelial cells, play a crucial role in cancer progression.⁵⁶ The presence of TG2 is widespread in endothelial cells and has been shown to impact tubule formation, leading to the suppression of angiogenesis and the progression of cancer.⁵⁷ However, research has demonstrated that the targeted and irreversible inhibition of TG2 transamination activity leads to the suppression of angiogenesis by controlling the release of VEGF into the

ECM, thereby activating signaling through VEGF receptor 2.⁵⁸ Furthermore, a separate investigation has revealed that reducing TG2 GTP-binding activity suppresses the subsequent NF- κ B/HIF1 α pathways, finally leading to the inhibition of angiogenesis.⁵⁹ In renal cell carcinoma, TG2 stimulates angiogenesis by breaking down the protein p53, resulting in the activation of HIF1 α and an increase in the production of VEGF.¹⁶ The ability of TG2 to either promote or inhibit angiogenesis is primarily determined by its cellular environment and structural configuration. Mechanical modifications in the vascular wall influence the process of angiogenesis, as constriction of blood vessels in the TME decreases the circulation of blood, resulting in hypoxia and promoting cancer progression.⁶⁰ TG2 modifies vascular stiffness by regulating the contractility and proliferation of smooth muscle cells.⁶¹ In addition, it affects the mechanical properties of collagen fibers in the vascular wall through cross-linking, thereby influencing matrix remodeling.⁶²

4.3. TG2-regulated signaling pathways

TG2 in tumor cells exerts both positive and negative effects. Both intracellular and extracellular TG2 may regulate cell signaling pathways that enhance cell survival, motility, adhesion, and invasive behavior. Previous studies have demonstrated that aberrant TG2 expression in epithelial cells leads to persistent activation of FAK, Akt, and NF- κ B.^{54,63} These pathways may promote cancer cell development by triggering EMT, enhancing treatment resistance and metastasis.⁶⁴ Constitutively active NF- κ B is a common feature of several late-stage malignancies, such as breast cancer. It upregulates the expression of numerous genes involved in inhibiting cell death, enhancing invasiveness, or promoting EMT and stem cell features.⁶⁵ Furthermore, the activation of NF- κ B is deemed crucial for inflammation-induced tumor progression. In breast cancer cells, NF- κ B controls the transcriptional regulation of TG2 through ATM signaling and by binding to two separate consensus sites in the TG2 promoter. The TG2 gene is suggested to act as a chromatin target for metastatic tumor antigen 1 and NF- κ B signaling in lipopolysaccharide-stimulated cells.⁶⁶ Research indicates that non-enzymatic scaffold TG2 may trigger NF- κ B through a unique non-canonical route by interacting with and breaking down the inhibitory protein I κ B α . TG2 expression has been shown to have an inverse correlation with the amount of I κ B α in breast tumors.⁶⁷ Stimulating the production of TG2 in breast cancer cells led to a reduction in I κ B α levels and an increase in NF- κ B activity.⁶⁸ Reducing TG2 in drug-resistant MCF-7 cells using RNA interference led to higher levels of I κ B α and reduced NF- κ B translocation into the nucleus.

5. Involvement of TG2 in EMT

A population of human epidermal cancer stem (ECS) cells derived from epidermal squamous cell carcinoma exhibits increased TG2 levels, migration, and invasion capabilities.⁶⁹ In addition, these cells exhibit increased expression of transcription factors commonly associated with EMT, such as Snail, Slug, Twist, and HIF-1 α , as well as mesenchymal structural proteins such as vimentin, fibronectin, and N-cadherin.⁷⁰ Consistent with a transition toward a mesenchymal phenotype, there is a reduction in the expression of E-cadherin, a hallmark associated with epithelial cells. Subsequent investigations have revealed that downregulation of TG2 significantly decreases the expression of EMT markers. This decrease in expression correlates with a reduced capacity of the cells to migrate and cover a scratch wound, as well as a decreased ability to invade Matrigel *in vitro*.

Nevertheless, it is crucial to consider the ramifications of administering a TG2 inhibitor as a therapeutic approach. NC9 functions as an irreversible inhibitor of TG2 by binding to its active site and inducing an open conformation of the enzyme. Administering NC9 therapy to ECSs leads to a reduction in the expression levels of Snail, Slug, and Twist.^{56,71} The downregulation of these transcription factors has been demonstrated to suppress E-cadherin expression.⁷² Furthermore, a decrease in the abundance of these transcription factors correlates with elevated levels of E-cadherin. Inhibition of TG2 by NC9 also results in decreased expression levels of vimentin, fibronectin, and N-cadherin. These alterations have been linked to diminished cell migration and decreased invasion capabilities, as studied using Matrigel.⁷²

5.1. Essential role of TG2 GTP binding activity in EMT

TG2 is a versatile enzyme capable of serving as a transamidase, GTP binding protein, protein scaffold, protein kinase, protein disulfide isomerase, and DNA hydrolase.⁷³ Among these functions, the transamidase and GTP binding activities are the two most extensively investigated.⁷⁴ To elucidate the activity of TG2 mutants necessary for inducing epithelial-to-mesenchymal transition (EMT), experiments were conducted to assess the ability of TG2 mutants to restore EMT in squamous carcinoma cells (SCC13)-TG2-shRNA2 cells. These cells express low levels of TG2, lack increased EMT marker expression, and demonstrate no associated biological responses. The results of these experiments demonstrate that wild-type TG2 can restore EMT marker expression, cell migration, and invasion on both plastic and Matrigel substrates.⁷⁰ Similarly, TG2 mutants TG2-C277S and TG2-W241A, which retain GTP binding activity, restore

EMT. In contrast, TG2-R580A, lacking GTP binding functionality, fails to reactivate the process of EMT. The findings underscore the pivotal role of GTP binding function in TG2-mediated induction of the EMT phenotype in ECS cells.⁷⁰ Furthermore, the importance of TG2 in maintaining stem cell viability in breast and ovarian cancer cells has been suggested.

5.2. NF- κ B signaling, TG2, and EMT

The involvement of NF- κ B in the process of EMT has been suggested across various types of cancer, including breast, ovarian, and pancreatic cancer. However, NF- κ B could have a distinct function in the development of epidermal squamous cell carcinoma. NF- κ B has been associated with keratinocyte dysplasia and hyperproliferation.⁷⁵ Nevertheless, previous studies have shown that suppressing NF- κ B activity might increase the susceptibility of mouse epidermis to carcinogenesis.⁷⁶ In a study conducted by Fisher and colleagues, it was found that TG2 levels were elevated in ECSs compared to non-stem cancer cells, while NF- κ B levels were reduced. In addition, a reduction in TG2 expression was found to correlate with increased NF- κ B levels. Furthermore, manipulating TG2 through knockdown or inhibition using NC9 did not alter the distribution of NF- κ B between the nucleus and cytoplasm.⁷⁰ In addition, increasing TG2 levels in spheroid culture led to a slight decrease in NF- κ B binding to the NF- κ B response element, as assessed through gel mobility supershift assessments. These molecular investigations provide compelling evidence indicating that NF- κ B does not mediate the actions of TG2 in ECS cells. Furthermore, suppressing NF- κ B-p65 in TG2-expressing cells does not lead to decreased expression of Snail, Slug, Twist, or mesenchymal marker proteins. In addition, simultaneous suppression of TG2 and NF- κ B does not result in a greater reduction in EMT marker protein levels compared to TG2 suppression alone.⁷⁰

The TGF- β , Notch, and Wnt/ β -catenin pathways play a key role in governing EMT, a process essential for cancer progression and metastasis.⁷⁷ TGF- β signaling may trigger EMT by activating transcription factors that suppress epithelial markers while enhancing mesenchymal characteristics. Notch signaling impacts EMT by interacting with other pathways, regulating cell differentiation and proliferation. Aberrant activation of the Wnt/ β -catenin pathway disrupts cell-cell adhesion and promotes invasive properties. TG2-mediated signaling interacts with several pathways, possibly amplifying or adjusting their impact on cell plasticity and migration. TG2 may influence the Wnt/ β -catenin pathway by influencing β -catenin stabilization and localization, thereby affecting gene transcription related to EMT and invasion.⁵¹ The TME, consisting of hypoxia

and ECM components, profoundly influences EMT. Under hypoxia conditions, HIFs may stimulate TG2 expression, thereby impacting cell-matrix interactions and facilitating EMT. The capacity of TG2 to cross-link ECM proteins modifies the physical properties of the ECM, creating a microenvironment conducive to EMT and tumor spread.³³ TG2 exhibits diverse functions in the TME and contributes to cancer development.

6. The role of TG2 in mediating tumor chemo-resistance

The persistent overexpression of TG2 in epithelial cancer cells triggers a multifaceted cascade of signaling pathways that actively foster resistance to chemotherapy and promote the acquisition of an invasive phenotype. For instance, the upregulation of TG2 expression in mammary epithelial cells may be attributed to the activation of multiple signaling pathways, including NF- κ B, Akt, FAK, and HIF.⁷⁸ Cancer cells exhibiting resistance to chemotherapy or derived from metastatic sites display increased expression of TG2.²⁶ TG2 significantly contributes to cellular resistance to chemotherapy, thereby contributing to oncogenic potential. This effect primarily stems from TG2's role in promoting the integration of fibronectin into the ECM, thereby affording protection to malignant cells against the effects of chemotherapy. In addition, TG2 has the potential to hinder the process of anoikis in damaged tissue cells by restoring integrin-dependent adhesion following injury, thereby further facilitating resistance to chemotherapy.⁷⁹

The downregulation of TG2 and the inhibition of endogenous TG2 using siRNA or small molecule inhibitors present potential avenues for reversing chemo-resistance and the invasive phenotype.⁸⁰ Such interventions hold promise for significantly improving the therapeutic effectiveness of anticancer drugs and inhibiting metastatic spread. In contrast, ectopic overexpression of TG2 has been observed to enhance the survival, motility, and invasive capabilities of cancer cells.⁶³ During apoptosis, a significant amount of Ca^{2+} is released into the intracellular space. Consequently, TG2 becomes activated while exhibiting its pro-crosslink activity. This activation enables TG2 to localize fragments of apoptotic cells, thereby preventing their recognition and subsequent phagocytosis by macrophages. The anti-apoptotic effect of TG2 has been shown to contribute to the induction of chemo-resistance in malignant cells.²⁶

6.1. Resistance in lung cancer

TG2 is one of the most prominently upregulated genes in lung cancer cells that have developed resistance to tumor necrosis factor-related apoptosis-inducing ligand

(TRAIL). The significant reduction of TG2 expression correlates with notable mitigation of TRAIL resistance and cell migration, suggesting TG2's involvement in these characteristics in TRAIL-resistant cells.²⁶ This resistance to TRAIL induced by TG2 is believed to occur through c-FLIP mediation, as inhibiting TG2 leads to a substantial decrease in c-FLIP levels. Moreover, the production of TG2 undergoes significant diminishment through the suppression of the epidermal growth factor receptor (EGFR), a process facilitated by the activation of JNK and ERK signaling pathways. TG2 is recognized as a potential molecular target for combating acquired chemo-resistance and metastasis in lung cancer. This pathway involves EGFR, MAPK (JNK and ERK), and cell migration.⁸¹

An intriguing approach for sensitizing p53-deficient lung cancer cells to TRAIL-induced apoptosis involves upregulating death receptor 5 through TG2 inhibition.¹⁴ TG2 not only regulates cellular responsiveness to the chemotherapy agent doxorubicin but also significantly contributes to the development of resistance to doxorubicin and cisplatin in breast and ovarian malignancies. The sensitivity of non-small cell lung cancer (NSCLC) cell lines to cisplatin appears to be heightened in cases where the TG2 gene promoter is methylated compared to cell lines expressing TG2. This finding implies a positive association

between cisplatin sensitivity and TG2 suppression. Analyzing the promoter methylation status of the TG2 gene may aid in identifying individuals who respond well to cisplatin in NSCLC. In addition, suppressing TG2 may represent a potentially useful method for enhancing the sensitivity of NSCLC to cisplatin.⁸² However, it is worth mentioning that, blocking the connection between TG2 and TOP2 α using glucosamine may be an effective approach to overcome resistance to radiation and other DNA damage-related therapies (Figure 2).

6.2. Resistance in breast cancer

Breast cancer cells exhibiting elevated levels of basal TG2 expression exhibit increased susceptibility to doxorubicin and reduced invasiveness compared to cancer cells with normal TG2 levels. Moreover, the chemoprevention group exhibits a higher TG2 expression in tumors.⁸³ Sublines characterized by elevated TG2 expression undergo a transition from a noninvasive to an invasive phenotype. Importantly, metastatic lymph nodes from breast cancer patients exhibit a notable increase in TG2 expression compared to the primary tumors from the same individuals. Elevated TG2 expression is observed in drug-resistant breast cancer cells with metastatic potential. This observation suggests that TG2 could serve as a useful

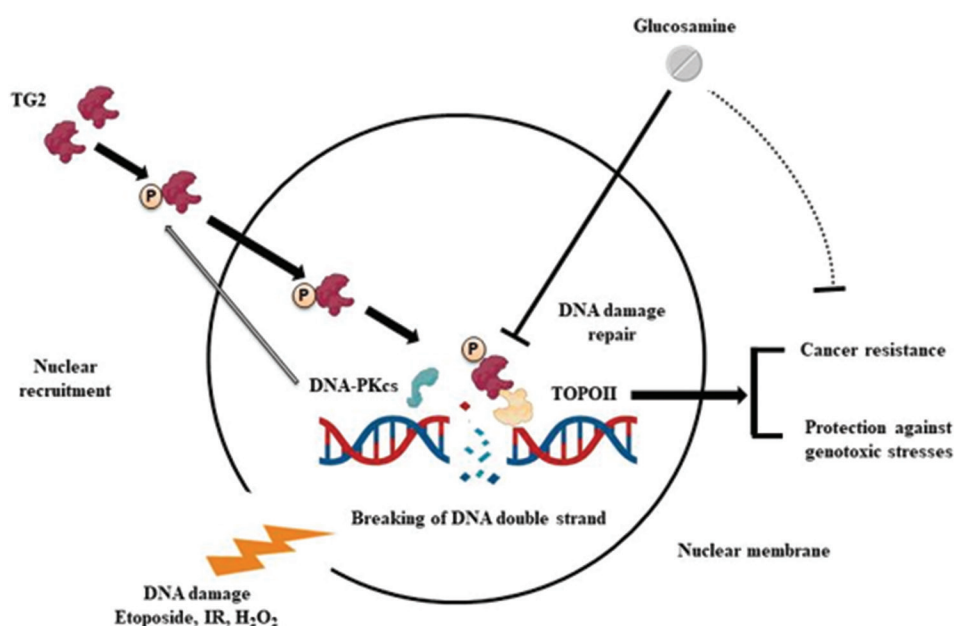


Figure 2. The role of TG2 in DSB repair. Upon the occurrence of significant DNA DSBs, TG2 is promptly activated through phosphorylation by DNA-PKcs. Subsequently, the phosphorylated TG2 translocates from the cytoplasm to the nucleus. Once in the nucleus, it is recruited or actively forms clusters at the sites of DNA DSBs and attaches to TOP2II α . This interaction between TG2 and TOP2II α is essential for DSB repair and enhances the effectiveness of radiotherapy in lung cancer.

Abbreviations: DNA-PKcs: DNA-dependent protein kinase catalytic subunit; DSB: Double-stranded break; IR: Ionizing radiation; TG2: Transglutaminase-2; TOP2II: Type II topoisomerase.

indicator for predicting these specific characteristics.⁸⁴ The inhibition of TG2 leads to an increased occurrence of doxorubicin-induced cell death in tumor cells.⁸⁵

The induction of apoptosis in drug-resistant cancer cells can be achieved by downregulating TG2 using cystamine or synthetic peptide R2. This downregulation restores the levels of I κ B α , resulting in the inactivation of NF- κ B. Inhibiting TG2 expression significantly increases the sensitivity of doxorubicin-resistant breast cancer cells to doxorubicin-induced apoptosis. TG2 facilitates the development of drug resistance by upregulating survival factors through the activation of NF- κ B.⁸⁶ RNA interference-mediated suppression of TG2 in drug-resistant cells leads to increased expression of I κ B α , causing NF- κ B translocation from the nucleus to the cytoplasm. TG2 activates NF- κ B through polymerization and the reduction of available I κ B α molecules in an inflammatory context. Thus, the upregulation of TG2 and subsequent induction of NF- κ B might potentially play a role in the development of drug resistance in breast cancer cells, independent of EGF signaling.⁶⁸

The precise mechanism by which TG2 expression is stimulated and facilitates the development of drug resistance remains incompletely elucidated. The potential contribution of TG2 to critical pathways governing multiple fundamental cancer characteristics might elucidate its involvement in treatment resistance (Table 2).

7. TG2 expression as a negative prognostic indicator

TG2 is widely recognized as a significant unfavorable prognostic factor, often associated with advanced disease stage, metastasis, and resistance to chemotherapy. In patients with renal cell carcinoma, there is a robust inverse correlation between TG2 expression and both disease-free survival and cancer-specific survival rates over a 5-year

period.⁹³ Moreover, individuals exhibiting elevated TG2 expression in colorectal cancer demonstrate a poorer overall survival rate compared to those with lower TG2 expression, suggesting that increased TG2 expression may serve as an independent adverse prognostic factor.⁹⁴ The loss of PTEN through TG2 mediation serves as a predictive factor for individuals diagnosed with stage II pancreatic ductal adenocarcinoma, irrespective of tumor stage, lymph node status, and tumor differentiation grade.⁹⁵ In the study conducted by Jin *et al.*, low expression of TG2 emerged as a noteworthy independent prognostic factor for improved overall survival in patients with laryngeal human SCCs who underwent adjuvant radiotherapy.⁹⁶ Furthermore, the expression of TG2 was found to be positively associated with another target of HIF-1 α , namely BNIP3.

The expression of TG2 in NSCLC, including subtypes other than adenocarcinoma such as SCCs, correlates strongly with the occurrence of recurrence. A notable association has been shown between high TG2 expression and decreased disease-free survival in NSCLC (hazard ratio [HR] = 1.554). This correlation is particularly pronounced in the non-adenocarcinoma subtype (HR = 2.184).²⁹ In the cohort of NSCLC patients undergoing treatment with the EGFR-tyrosine kinase inhibitor (TKI) therapy, those with low TG2 expression exhibit a significantly prolonged progression-free survival (PFS) compared to those with high TG2 expression. Similarly, among individuals with wild-type EGFR who received EGFR-TKI treatment, patients exhibiting decreased TG2 expression levels experience extended PFS duration. Thus, the expression of TG2 has the potential to serve as a predictive factor for PFS in NSCLC patients undergoing EGFR-TKI treatment.⁹⁷

The expression of TG2 in the primary tumor of patients with advanced breast cancer exhibits an adverse correlation with both recurrence-free survival and distant metastasis-free survival (DMFS). Concurrent upregulation

Table 2. Transglutaminase-2-mediated drug resistance

Drug	Tumor type	Mechanism	References
Doxorubicin	Breast, non-small cell lung cancer, glioma	Activation of survival pathways (NF- κ B, Bcl2, AKT, FAK, EGF, JNK)	87
TRAIL	NCLC	Activation of survival pathways (NF- κ B, Bcl2, AKT, FAK, EGF, JNK)	81
BCNU	Glioblastoma	Inhibition of apoptosis (Bax, DR5, survivin, Bim, Bad, cFLIP)	88
Doxorubicin	Breast	Alteration of extracellular matrix proteins	89
Cisplatin and dacarbazine	Melanoma	Alteration of extracellular matrix proteins	90
Doxorubicin	Breast	Gene regulation	91
Doxorubicin	Breast, ovarian	Induction of EMT	92

Abbreviations: TRAIL: Tumor necrosis factor-related apoptosis-inducing ligand; NF- κ B: Nuclear factor kappa B; FAK: Focal adhesion kinase; EGF: Endothelial growth factor; JNK: Jun N-terminal kinase; EMT: Epithelial-mesenchymal transition.

of TG2 and IL-6 correlates with a shortened duration of DMFS in breast cancer patients, suggesting TG2's role as a mediator of distant metastasis alongside IL-6.⁹⁸ Invasive ductal carcinomas (IDCs) of the breast presenting an accumulation of stromal TG2 demonstrate significantly lower disease-free survival compared to those with low TG2 expression. Furthermore, stromal TG2 accumulation serves as an independent risk factor for recurrence, indicating that overexpression of TG2 in the tumor stroma could potentially be used as an unfavorable prognostic indicator for breast IDC.⁹⁹ Nevertheless, a study conducted in Greece has demonstrated a potentially positive impact of TG2 expression on overall survival. In addition, TG2 appears to act as an independent and favorable prognostic factor for survival, potentially by enhancing the apoptotic response to chemotherapy.¹⁰⁰

Comparing the prognostic implications of TG2 with other biomarkers requires an examination of its involvement in cancer progression, treatment resistance, and overall patient survival. TG2 is associated with a negative prognosis due to its involvement in EMT and chemo-resistance. Similarly, biomarkers such as HER2 in breast cancer and EGFR mutations in lung cancer serve as crucial prognostic indicators,¹⁰¹ each offering distinct implications for tumor behavior and response to treatment. To understand the predictive power of TG2, it is crucial to compare its predictive abilities with those of other biomarkers. HER2 overexpression or EGFR mutations may indicate particular treatment options and resistance patterns,¹⁰² while the involvement of TG2 in cellular processes such as EMT and stemness may reveal a broader mechanism of tumor aggressiveness and unfavorable prognosis. This comparative analysis will provide a thorough examination of how TG2 and other biomarkers impact cancer prognosis, providing insights into their potential as therapeutic targets or indicators of disease progression.

8. Strategies for targeting TG2 in anti-tumor approaches

The development of novel anti-cancer treatments could benefit from translational research exploring the intricate relationship between TG2 expression, its function, and the location and behavior of cancers. In addition, a deeper understanding of the multifunctional nature of TG2 further enriches this avenue of investigation.²⁶ Elevated TG2 levels in cancer present a promising target for therapy through the use of targeted inhibitors. Specifically, targeting cancer cells using TG2 and glutaminase-1 (GLS1) inhibitors holds the potential for inducing synthetic cellular lethality. Notably, combined inhibition of TG2 and GLS1 induces cell death,

while individual treatment with either compound exhibits minimal effect on cell death. Thus, the combination of these inhibitors emerges as an effective approach in the combat against cancer.¹⁰³

Resveratrol exhibits the potential to inhibit the migration of cells expressing TG2. Notably, during the migration process, the presence of resveratrol enhances the immunoreactivity of TG2 in migrating cells without causing any changes in the overall quantity of TG2 protein. In addition, this effect is further augmented in the presence of elevated levels of Ca^{2+} .²⁶ The reduction of intracellular Ca^{2+} levels using a Ca^{2+} chelator attenuates the increased TG2 immunoreactivity induced by resveratrol. In native polyacrylamide gel, the presence of a non-phosphorylated TG2 protein form in cells treated with resveratrol is associated with a decelerated migration rate. This particular form of TG2 is exclusively localized in plasma membrane fractions and is modulated by intracellular Ca^{2+} concentration. These findings underscore the essential role of Ca^{2+} in the regulation of TG2-mediated cell migration.³⁰

Cardamonin effectively reduces the expression of TG2 in sarcoma cells and directly hinders the action of TG2. The suppression of the TG2 gene results in the inhibition of sarcoma cell invasion and migration, alongside a decrease in MMP2 and MMP9 activity. Notably, cardamonin holds the potential to thwart the migration of cancer cells characterized by elevated TG2 levels.¹⁰⁴ This discovery paves the way for a novel pharmacophore concept aimed at developing potent TG2 inhibitors. In a similar vein, cantharidinate exhibits the capability to suppress the TG2 expression in human colorectal cancer.¹⁰⁵ Moreover, GK13, identified as a competitive inhibitor of TG2, effectively suppresses TG2-mediated polymerization of $\text{I}\kappa\text{B}\alpha$, with the inhibition being dose-dependent. Furthermore, GK13 exhibits superior effectiveness in cancer treatment compared to doxorubicin.¹⁰⁶

In ovarian cancer, ITP-79 hinders the attachment of a TG2 peptide to a 42-kDa segment of fibronectin in a dose-dependent manner. In addition, it decreases TG2 stabilization, imitating the action of GTP, a negative allosteric regulator of TG2 function. These observations indicate that ITP-79 may employ an allosteric mechanism, as it influences distant target sites.¹⁰⁷ Furthermore, the silencing of TG2 using siRNA reduces the adhesion and motility of HeLa cells by reducing the phosphorylation of the protein kinase Akt and reactive oxygen species. Conversely, TG2 overexpression exerts the opposite effects. These findings underscore the potential of TG2 as a valuable target for the development of therapeutic anti-cancer vaccines.¹⁰⁸

9. Conclusion

A substantial body of evidence has been assembled, substantiating the presence and functionality of TG2 within the TME. Furthermore, there is an increasing understanding of the underlying mechanisms through which TG2 facilitates cancer progression, operating at both cellular and extracellular levels. TG2 remains a promising candidate for cancer therapy, owing to its dual role in regulating cellular behavior within the TME, facilitating ECM cross-linking, and modulating biophysical and biomechanical stresses. The growing comprehension of the dynamic function of TG2 in cancer development and progression, coupled with extensive research into mechanical processes contributing to cancer cell survival and invasion, suggests that preclinical studies focusing on a specific timeframe could establish TG2 as a viable and valuable intervention target, both biological and biomechanical aspects. This approach could broaden treatment options for cancers, particularly advanced-stage tumors characterized by ECM deposition and remodeling, which currently carry a bleak prognosis. However, one significant concern regarding TG2 revolves around its perceived high prognostic and therapeutic efficacy. Therefore, gaining a deeper understanding of the pertinent genetics and epigenetics is of utmost importance to discern TG2 as therapeutic targets tailored to individual patients.

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The authors declare no conflict of interest.

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