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INVITED REVIEW

Tissue transglutaminase: a new target to reverse cancer drug resistance

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Abstract Cancer resistance mechanisms, which result from intrinsic genetic alterations of tumor cells or acquired genetic and epigenetic changes, limit the long-lasting benefits of anti-cancer treatments. Tissue transglutaminase (TG2) has emerged as a putative gene involved in tumor cell drug resistance and evasion of apoptosis. Although some reports have indicated that TG2 can suppress tumor growth and enhance the growth inhibitory effects of anti-tumor agents, several studies have presented both pro-survival and anti-apoptotic roles for TG2 in malignant cells. Increased TG2 expression has been found in several tumors, where it was considered a potential negative prognostic marker, and it is often associated with advanced stages of disease, metastatic spread and drug resistance. TG2 mediates drug resistance through the activation of survival pathways and the inhibition of apoptosis, but also by regulating extracellular matrix (ECM) formation, the epithelial-to-mesenchymal transition (EMT) or autophagy. Because TG2 knockdown or inhibition of TG2 enzymatic activity may reverse drug resistance and sensitize cancer cells to drug-induced apoptosis, many small molecules capable of blocking TG2 have recently been developed. Additional insight into the multifunctional nature of TG2 as well as translational studies concerning the correlation between TG2 expression, function or location and cancer behavior will aid in translating these findings into new therapeutic approaches for cancer patients.

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Introduction

Despite advances in our understanding of the molecular mechanisms of tumor development, prevention and treatment, the long-term survival for patients with recurrent/advanced disease remains low. Resistance to chemotherapy as well as the genetic heterogeneity of tumors is the leading causes of limited activity in anti-cancer strategies. Although higher response rates have been achieved using the latest poly-chemotherapy regimens and molecular targeted compounds, tumor resistance mechanisms resulting from intrinsic genetic alterations of tumor cells or acquired genetic and epigenetic changes limit the long-lasting benefits of anti-cancer treatments.

Tumor progression and metastasis requires cancer cells to circumvent stress conditions, such as hypoxia or lack of nutrients, and escape the immune system attack. This selective pressure facilitates metastatic potential by activating tumor survival mechanisms against the pro-apoptotic signals induced by physiological and pharmacological conditions. Indeed, metastatic tumors are highly resistant to chemotherapy drugs, and tumors resistant to chemotherapy show more aggressive metastatic phenotypes. Over the past 20 years, many genes that may be involved in the mechanisms of drug resistance have been identified. Among these, several studies have indicated tissue transglutaminase, also defined as transglutaminase 2 (TG2), as a putative gene involved in tumor drug resistance and evasion of apoptosis.

TG2 is a member of the larger family of transglutaminases and is a multi-domain, multifunctional enzyme

that is ubiquitously expressed in mammalian tissues and involved in a variety of cellular processes. TG2 catalyzes Ca^{2+}-dependent post-translational protein modifications by inserting irreversible ε-(γ-glutamyl)-lysine cross-links between polypeptide chains. TG2 is also known to have guanosine-5-triphosphate hydrolase, protein disulfide isomerase and protein kinase activities (Mehta et al. 2010; Park et al. 2010a). Although predominantly localized within the cytosol, nucleus and cellular membrane compartments, TG2 can also be secreted outside the cell. Consequently, TG2 has been associated with several biological functions including signal transduction, apoptosis, cell adhesion, cell migration and extracellular matrix (ECM) formation (Mehta et al. 2010; Park et al. 2010a).

TG2 has been described as a promoter or an antagonist of apoptosis depending on the relevant cellular context. Although some reports have indicated that TG2 can suppress tumor growth and contribute to the growth inhibitory effects of anti-tumor agents, several studies support a pro-survival and anti-apoptotic role for TG2 in malignant cells.

TG2 exerts its anti-apoptotic effects through different mechanisms involving both its transamidation and GTP-binding activities. For instance, TG2-induced protein cross-linking protects tumor cells from caspase cleavage and promotes NFκB-dependent cell survival (Kim et al. 2006; Mann et al. 2006; Yamaguchi and Wang 2006; Verma and Mehta 2007; Jang et al. 2010a). Moreover, TG2-mediated transamidation of RB in the nucleus protects this oncogenic protein from degradation, thereby promoting survival (Boehm et al. 2002). Furthermore, the association of TG2 with some members of the integrin family promotes the anchoring of cells to the ECM and activates cell survival pathways (Akimov et al. 2000; Herman et al. 2006; Mangala et al. 2007).

Some of these mechanisms will be further described in the following sections. In detail, we will provide potential mechanistic explanations for the association between TG2 induction and the emergence of cancer drug resistance and suggest how TG2 targeting may potentiate anti-cancer strategies.

TG2 and cancer aggressiveness

TG2 overexpression has been observed in pancreatic (Iacobuzio-Donahue et al. 2003), breast (Grigoriev et al. 2001; Mehta et al. 2004), colon (Miyoshi et al. 2010), ovarian (Satpathy et al. 2007) and non-small cell lung cancers (NSCLC) (Martinet et al. 2003), as well as in glioblastoma (Zhang et al. 2003) and melanoma (Fok et al. 2006). Increased TG2 expression has been considered a potential negative prognostic marker and is often associated with advanced stages of disease, metastatic spread and drug resistance (Zhang et al. 2003; Mehta et al. 2004, 2006; Fok et al. 2006; Herman et al. 2006).

Cell invasion is critical to cancer metastasis and involves a phenotypic switch and an extensive remodeling of the ECM by matrix metalloproteinases (MMPs). The importance of TG2 in ECM deposition and stabilization is now well established and supports studies that have demonstrated the involvement of TG2 in wound healing and inflammation (Chau et al. 2005; Collighan and Griffin 2009; Fisher et al. 2009; Mehta et al. 2010). Notably, chronic inflammation can lead to cancer development. TG2 can bind the gelatin-binding domain of fibronectin with high affinity through its N-terminal β-sandwich domain and enhance the association of fibronectin and integrins on the cell surface. In this way, TG2 can influence several aspects of cancer cell behavior, including motility, invasion, growth, and survival (Akimov et al. 2000; Chau et al. 2005; Mangala et al. 2007; Collighan and Griffin 2009; Fisher et al. 2009; Mehta et al. 2010). TG2 can act either by direct cross-linking or by its GTP-binding site that mediates signal transduction via phospholipase C, focal adhesion kinase and PI3K activation (Mehta et al. 2010).

Recently, it has been demonstrated that aberrant expression of TG2 is sufficient for inducing the transdifferentiation of mammary epithelial cells into mesenchymal cells, a process known as the epithelial-to-mesenchymal transition (EMT), which is required during embryonic development and is associated with increased tumor aggressiveness and metastatic potential (Kumar et al. 2010). TG2-induced EMT confers invasiveness, drug resistance and a tumorigenic phenotype, and some authors have suggested that this finding establishes a strong link between TG2 expression and the progression of metastatic breast disease (Kumar et al. 2010). Similarly, it has been demonstrated that ovarian cancer cells expressing TG2 adopt a mesenchymal phenotype characterized by the loss of epithelial markers and an increase in invasive behavior. This mechanism seems to be mediated by activation of the NFκB complex via TG2. Moreover, the TG2-dependent induction of EMT in an orthotopic xenograft ovarian model led to increased tumor formation, peritoneal metastases and malignant ascites (Shao et al. 2009).

Finally, a recent study provided first-time evidence demonstrating that sustained expression of TG2 conferred stem cell-like properties in non-transformed and transformed mammary epithelial cells, while downregulation of TG2 attenuated stem cell properties in both populations. Taken together, these results suggest a new function for TG2 and reveal a novel mechanism responsible for promoting stem cell characteristics in adult mammary epithelial cells (Kumar et al. 2011).

TG2 and drug resistance

As reported above, high levels of TG2 expression were observed in drug-resistant cancer cells. Interestingly, TG2 knockdown or TG2 enzymatic inhibitors may reverse drug resistance and sensitize cancer cells to stress- or drug-induced apoptosis (Antonyak et al. 2004; Choi et al. 2005; Yuan et al. 2005, 2007; Herman et al. 2006; Kim et al. 2006; Cao et al. 2008; Verma et al. 2008a). It has also been suggested that TG2 overexpression is a general mechanism for the induction of drug resistance independent of multi-drug resistance (Han and Park 1999a). However, one study recently demonstrated that increased TG2 expression conferred drug resistance to some but not all chemotherapeutics in glioma cells, indicating that the induction of TG2 was not a general mechanism of drug resistance (Dyer et al. 2011).

The exact mechanism through which TG2 expression is induced and mediates drug resistance is not completely understood. The involvement of TG2 in many important pathways that regulate several hallmarks of cancer may explain its role in drug resistance (Table 1).

Several studies have reported a straightforward relationship between TG2 expression and doxorubicin drug resistance. Han and colleagues demonstrated that the generation of hydrogen peroxide induced by doxorubicin treatment was one of the key factors in enhancing TG2 expression in lung cancer cells (Han and Park 1999b). Proteomic analysis (Park et al. 2007) and epigenetic profiling (Chekhun et al. 2007) studies of doxorubicin-resistant breast cancer cells confirmed the role of TG2 as a target protein to increase doxorubicin resistance. Another mechanism of TG2 induction involves the activation of the EGF signaling pathway, which may contribute to the oncogenic potential of breast cancer cells by promoting chemoresistance toward doxorubicin and other drugs (Antonyak et al. 2004). Moreover, under stress conditions, TG2 expression can be regulated by the activation of several different pathways as well as by transcription factors, such as HIF-1 or NFκB (Jang et al. 2010a; Mehta et al. 2010), or epigenetic mechanisms (Park et al. 2010b; Dyer et al. 2011).

TG2 overexpression in various cancer cell types is often associated with constitutive activation of NFκB (Verma and Mehta 2007). Moreover, the inhibition of TG2 activity by specific inhibitors can down-regulate NFκB activity (Kim et al. 2006; Cao et al. 2008). Several signaling pathways implicated in cancer likely lead to the activation of NFκB, a family of transcription factors that activates genes responsible for cell proliferation, survival, angiogenesis and metastasis. In many cancers, chemotherapy also induces constitutive activation of NFκB, thereby making the tumor refractory to the treatment. The main regulation of NFκB occurs through association with the inhibitor IκB family of proteins. In response to stimuli, NFκB-bound IκBα is phosphorylated and then degraded by the proteasome, which results in the release of NFκB dimers that translocate into the nucleus, where they bind response elements to activate target genes. The p65 subunit may be further altered by post-translational modifications such as phosphorylation, acetylation or methylation, all of

Table 1 Drug resistance mediated by TG2 in tumors

Mechanism	Drug	Tumor type	References
Activation of survival pathways (NFkB, Bcl2, BclXl, EGF, JNK, ERK, AKT, FAK)	Doxorubicin	Breast, glioma, NSCLC	(Antonyak et al. 2004; Kim et al. 2006, 2009; Park et al. 2010b; Dyer et al. 2011)
	Cisplatin	Ovarian, NSCLC	(Cao et al. 2008; Park et al. 2010b)
	TRAIL	NCLC	(Li et al. 2011)
	Gemcitabine	Pancreas	(Verma et al. 2008a)
	Retinoic acid		(Antonyak et al. 2003)
	HDAC-Is	Breast, head and neck, colon	Carbone and Budillon, unpublished data
Inhibition of apoptosis (Bax, DR5, survivin, Bim, Bad, cFLIP)	Doxorubicin	Breast	(Park et al. 2009)
	BCNU, Radiation	Glioblastoma	(Yuan et al. 2005, 2011)
	TRAIL	NSCLC, renal	(Frese-Schaper et al. 2010; Jang et al. 2010b)
Alteration of ECM proteins	Doxorubicin	Breast	(Herman et al. 2006)
	Cisplatin and dacarbazine	Melanoma	(Fok et al. 2006)
	BCNU	Glioblastoma	(Yuan et al. 2007)
Regulation of autophagy		Pancreas	(Akar et al. 2007)
Induction of EMT	Doxorubicin	Breast, ovarian	(Shao et al. 2009; Kumar et al. 2010)
Gene regulation	Doxorubicin	Breast	(Chekhun et al. 2007; Park et al. 2007)

which affect its function and binding to co-activators or repressors. The capability of TG2 to increase NFκB activity has been described in breast cancer cells resistant to doxorubicin (Kim et al. 2006). Interestingly, the induction of TG2-mediated NFκB activity was observed in both EGFR-positive and EGFR-negative breast cancer cells (Kim et al. 2006). The involvement of NFκB in TG2-mediated cisplatin resistance was reported in epithelial ovarian cancer cells, where a cytotoxic synergistic interaction between the TG2 inhibitor KCC009 and cisplatin demonstrated that the enzymatic activity of TG2 was important for modulating NFκB function (Cao et al. 2008). Our group has recently demonstrated that vorinostat, an HDAC inhibitor, induces TG2 expression in cancer cell lines from different tissues of origin and that this induction represents a mechanism of resistance against the anti-tumor effects of vorinostat.[1] We have also shown the existence of a vorinostat-induced NFκB-dependent loop mediating TG2 induction and resistance.

TG2, through its functional and structural complexity, contributes to the regulation of NFκB in different ways. TG2 can form a direct complex with NFκB p50/p65 dimers in the cytoplasm and modify its affinity for IκBα (Verma and Mehta 2007). It has also been shown that IκBα is a good substrate for TG2 and that linkage of these proteins can induce the creation of an insoluble cytosolic polymer that is unable to bind and sequester NFκB in the cytosol (Lee et al. 2004). TG2 can also associate with p65 in the nucleus and redirect it to non-canonical targets, including the TG2 gene itself, leading to the formation of a positive feedback loop in resistant cells (Verma and Mehta 2007). Moreover, p65 serves as substrate for TG2 serine–threonine kinase activity and can be phosphorylated by TG2 at Ser536, which is required for transactivation activity (Verma and Mehta 2007).

Recently, Li et al. demonstrated that TG2 mediates tumor necrosis factor-related apoptosis-inducing factor (TRAIL) resistance and cell migration through c-FLIP and MMP-9. They showed EGFR-mediated TG2 expression via JNK and ERK, but not AKT and NFκB, confirming the existence of multiple mechanisms that mediate TG2-induction in resistant cells (Li et al. 2011). Interestingly, the TG2 inhibitor KCC009 reversed resistance to TRAIL through the up-regulation of death receptor 5 (DR5) in lung cancer cells, independent of NFκB and p53 (Frese-Schaper et al. 2010).

Other drug resistance mechanisms may involve the capability of TG2 to promote degradation of the phosphatase PTEN, resulting in the constitutive activation of the FAK/AKT cell survival pathway and resistance to gemcitabine, which can be reversed by knocking down TG2 expression (Verma et al. 2006, 2008b). TG2 overexpression downregulates the susceptibility of doxorubicin-resistant breast cancer cells to drug-induced apoptosis and also decreases the expression of cell survival factors such as Bcl2 and BclXL via NFκB (Kim et al. 2009), but also inhibits accumulation of cytosolic nucleophosmin, an anti-apoptotic protein that binds Bax and decreases its levels (Park et al. 2009). Inhibition of TG2 activity by KCC009 or KCA075 results in the sensitization of glioblastoma cells to (*N*,*N*′-bis(2-chloroethyl)-*N*-nitrosourea, carmustine (BCNU) by changing the levels and activity of pro- and anti-apoptotic proteins, such as Bim, Bad and GSK-3β (Yuan et al. 2005).

In addition to activation of survival pathways or inhibition of apoptosis, TG2 also mediates chemoresistance by regulating ECM proteins. As reported above, TG2 may contribute to doxorubicin resistance in breast cancer cells by promoting interactions between integrins and fibronectin and activating cell survival signaling pathways (Herman et al. 2006). To support this mechanism, it was shown that interference of the interaction between TG2 and fibronectin in the ECM by the TG2 inhibitor KCC009 enhances the sensitivity of glioblastoma cells to BCNU (Yuan et al. 2007). In melanoma cells, TG2 expression promotes integrin-mediated cell survival signaling pathways, resulting in cisplatin and dacarbazine resistance (Fok et al. 2006).

As reported above, TG2 can promote EMT as well as stem cell characteristics. Recent evidence now indicates that tumor cell EMT not only causes increased metastasis, but also contributes to drug resistance. EMT reflects the emergence of chemorefractory cells with stem cell-like features and also seems to play a role in establishing the resistance to molecular anti-cancer compounds such as EGFR targeting agents or anti-VEGF agents (Bruzzese et al. 2011; Carbone et al. 2011). Additionally, TG2-induced EMT may result from the constitutive activation of NFκB, confirming a crucial role for this pathway in TG2-mediated chemoresistance and tumor aggressiveness (Mehta et al. 2010). More debated is the role of the cross-talk between TG2 and a potent inducer of EMT such as TGFβ (Mehta et al. 2010).

Recently, it has also been shown that TG2 may regulate autophagy (Akar et al. 2007; D'Eletto et al. 2009). Autophagy is a homeostatic and catabolic process by which cells consume parts of themselves to survive starvation and stress. Autophagy is also a mechanism of stress tolerance that maintains cell viability and can lead to tumor dormancy, progression and therapeutic resistance. However, in some contexts, excessive or prolonged autophagy can lead to tumor cell death. TG2 catalyzes the final steps in autophagosome formation during autophagy (D'Eletto et al. 2009). However, the induction of TG2 by PKCδ results in the suppression of autophagic cell death in pancreatic

[1] Carbone and Budillon, unpublished data.

Table 2 Small molecule inhibitors of TG2

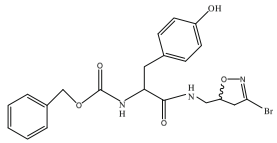
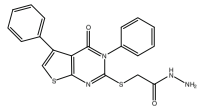
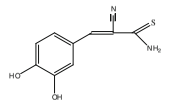
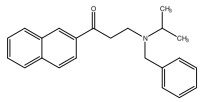
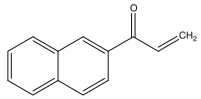
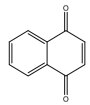
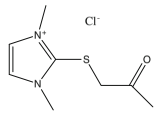
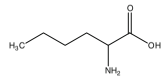
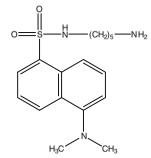
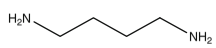
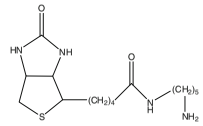
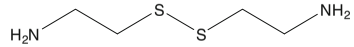
	Compound	Structure	Specificity	IC_{50} values (in vitro assay)	Mechanism of action	Application in experimental disease models
Irreversible inhibitors	KCC009		Specific	1–10 μM	Inhibition of catalytic triad	Cancer: ovarian, glioblastoma, non-small cell lung, melanoma, breast, colon, meningiomas
	KCA075		Specific	Unknown		
	Iodoacetamide		Not specific	0.1–1 mM	Disulfide bonding formation	Neurodegenerative disease
Reversible inhibitors	Thieno[2,3-d]pyrimidin-4-one acylhydrazide (LDN-27219)		Specific	Unknown	Competition of GTP-binding site	Neurodegenerative disease
	Tyrphostin 47		Specific	3 μM	Competition of GTP-binding site	Cancer: small cell lung, ovarian, breast, pancreatic, lymphoma; neurodegenerative disease
	ZM39923		Not specific	0.01 μM	Disulfide bonding formation, competition of GTP-binding site	Neurodegenerative disease
	ZM449828		Not specific	0.005 μM		Neurodegenerative disease
	Naphthoquinone or VitK3		Specific	0.015 mM, complete inhibition	Inhibition of catalytic triad	Neurodegenerative disease
	1,3-dimethyl-2-[(2-oxopropyl)thio]imidazolium		Not specific	0.004 mM	Pan-transglutaminase inhibition	Celiac sprue

Table 2 continued

	Compound	Structure	Specificity	IC_{50} values (in vitro assay)	Mechanism of action	Application in experimental disease models
Competitive amino inhibitors	Norleucine		Not specific	0.018 mM	Competitive amine inhibitors	Celiac sprue
	MDC		Specific	Unknown	Competitive amine inhibitors	Cancer: colon, non-small cell lung, melanoma, breast, ovarian, glioblastoma
	Putrescine		Specific	Unknown	Competitive amine inhibitors	Cancer: neuroblastoma
	5-(biotinamido)pentylamine		Specific	Unknown	Competitive amine inhibitors	Cancer: colon, neuroblastoma
	Cystamine		Not specific	0.022 mM	Disulfide bonding formation, competition of GTP-binding site, competitive amine inhibitor	Cancer: colon, breast, non-small cell lung, liver, glioblastoma; neurodegenerative disease

cancer cells that are frequently insensitive to standard chemotherapeutic agents (Akar et al. 2007). It has been suggested that the transamidating activity of TG2 in particular is a key regulator of cross-talk between autophagy and apoptosis (Rossin et al. 2011).

TG2 targeting

Overall, reported data indicate that TG2 can represent a marker for poor patient prognosis and that its expression correlates with resistance to treatment and tumor metastasis in both animal models and human cancers. Therefore, it is increasingly clear that TG2 is a novel putative therapeutic target for the treatment of resistant or advanced tumors. Because increasing evidence has suggested that TG2 is involved in numerous diseases such as inflammation and cancer and those of the central nervous system, many small molecules capable of blocking the activity of this protein have been recently developed.[2]

The TG2 inhibitors developed thus far can be divided in three classes based on their mechanisms of inhibition; only a few have been used in cancer models, and none have been used in patients (Siegel and Khosla 2007). The most frequently used and best defined for their potential anti-tumor effects are summarized in Table 2. Irreversible inhibitors such as KCC009 or KCA075 prevent enzyme activity by covalently modifying the enzyme, thereby preventing substrate binding. These inhibitors, as reported in the previous sections, have been used to enhance both in vitro and in vivo chemotherapy in several preclinical cancer models, such as ovarian, non-small cell lung, melanoma, breast and colon cancers as well as glioblastoma and meningioma (Yuan et al. 2005, 2007, 2008, 2011; Cao et al. 2008; Satpathy et al. 2009; Frese-Schaper et al. 2010). Reversible TG2 inhibitors block substrate access to the enzyme active site without covalently modifying the enzyme. The competitive amine inhibitors block TG2 activity by competing with natural amine substrates, such as protein-bound lysine residues, in the transamidation reaction. One such agent that is commonly used to inhibit TG2 activity is monodansylcadaverine (MDC), one of the first compounds used in experimental models. Structural similarity with the lysine side chain allows MDC to be used not only as an amine donor for the fluorescence incorporation assay of TG2 activity, but also as a competitive substrate to inhibit cross-linking of natural substrates (Yuan et al. 2005). Cystamine is not a specific inhibitor, because it can act as an amine competitor, an inducer of TG2 disulfide bonds and a GTP-binding compound. Recent findings suggest that cystamine may be an effective sensitizer of TRAIL-induced apoptosis (Jang et al. 2010b).

On the other hand, Metha et al. (2010) suggested that the transamidation activity of TG2 was not involved in the EMT process, chemoresistance or metastasis. These authors suggested alternate approaches to downregulate TG2 expression, such as the application of small interfering RNA (siRNA) oligonucleotides rather than TG2 inhibitors. Indeed, TG2 siRNA was successfully delivered to orthoptopically growing pancreatic tumors in nude mice and significantly enhanced the therapeutic efficacy of gemcitabine (Verma et al. 2008a). However, although these latter approaches have been successfully used in preclinical models both in vitro and in vivo, clinical evidence for the effectiveness of this therapeutic approach is modest and several concerns for their application in patients can be raised (Chen and Zhaori 2011).

Conclusions

The role of TG2 in tumors is still controversial because it might promote or suppress apoptosis or tumor growth. Moreover, although we summarized the evidence suggesting that TG2 can be considered a good target to reverse drug resistance, several reports have suggested that transcriptional activation of TG2 might, on the contrary, contribute to the growth inhibitory effect of several anti-tumor agents (Esposito et al. 2003; Palmieri et al. 2007; Lentini et al. 2009). Notably, TG2 induction can play opposite roles for the same chemotherapeutic agent depending on the context. A typical example is retinoic acid (RA), a potent activator of TG2. TG2 was identified as a direct RA target gene having a functional retinoid response element in its promoter (Nagy et al. 1996). TG2 expression was induced by RA in human pancreatic cancer cells, and its inhibition partially reversed the antiproliferative effect of RA (El-Metwally et al. 2005). Moreover, it was demonstrated that induction of TG2 by RA through the PML-RAR signaling pathway induced differentiation of acute promyelocytic leukemia (Benedetti et al. 1996). On the other hand, RA-mediated expression of TG2 also induced increased migration and invasion (Joshi et al. 2006). Other evidence has suggested that TG2 may serve as a survival factor and is induced by RA via a mechanism involving PI3K, which is antagonized by the Ras-ERK pathways (Antonyak et al. 2003). Thus, TG2 functions are dictated by its cellular location, interaction with other proteins and environmental or disease context. Cytosolic TG2 shows only latent transamidating activity due to low Ca^{2+} inside the cells and is mainly involved in signal transduction pathways, but can be activated and participate in the cellular response to extreme

[2] http://www.brenda-enzymes.org/php/result_flat.php4?ecno=2.3.2.13.

stresses such as hypoxia, nutrient deprivation or in response to chemotherapeutic agents.

Interestingly, in addition to the complex protein structure, recent studies have suggested that two structurally distinct TG2 protein isoforms, the full-length (TG2-L) and short-length (TG2-S), form that result from alternative splicing and exert different effects on cell survival and differentiation (Antonyak et al. 2006; Tee et al. 2010). Both isoforms retain transamidation activity, but the short isoform lacks the residual GTP-binding and carboxy-terminal portion for the recognition and binding to phospholipase C. The TG2-L isoform confers a strong survival advantage to cells, whereas TG2-S is pro-apoptotic. Interestingly, the ability of TG2-S to induce cell death is not dependent on transamidation, but rather on its unusual ability to undergo high-order aggregations and consequently to induce inappropriate protein oligomerization, an increasingly common mechanism for inducing cell death (Antonyak et al. 2006). Furthermore, overexpression of TG2-S or of the GTP mutant of TG2-L as well as repression of TG2-L expression or of its transamidase activity induced differentiation in neuroblastoma cells (Tee et al. 2010). Other studies are needed to demonstrate that the controversial role of TG2 we have described could be ascribed to distinct expression of the two isoforms. However, these findings are particularly intriguing and challenging, suggesting the selection of isoform-specific inhibitors from a therapeutic point-of-view.

In conclusion, additional insights into the multifunctional nature of TG2 as well as translational studies on the correlation between TG2 expression, function or location and cancer behavior might help to translate TG2 targeting from the bench to bedside.

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Conflict of interest The authors have no conflicts of interest to declare.

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