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REVIEW ARTICLE

Biological and therapeutic significance of tissue transglutaminase in pancreatic cancer

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Abstract Pancreatic ductal adenocarcinoma (PDA) is one of the deadliest cancers world-wide with an estimated annual incidence and mortality rates of approximately 6,500 cases in the UK, over 40,000 cases in Europe, 19,000 cases in Japan and over 30,000 cases in the United States. Difficulty to diagnose the disease at an early stage, rapid progression and intrinsic resistance to currently available therapies are major factors that contribute to poor disease outcome in these patients (overall 5 years survival, <3%). Identification of cancer cell-encoded genes that contribute to the development of intrinsic resistance and metastatic spread of the PDA tumors, may yield immediate clinical benefits in terms of revealing new therapeutic targets for effective treatment of the disease. This article discusses the significance of tissue-type transglutaminase (TG2) whose expression is elevated in the majority of PDA tumors and cell lines. Based on the published data and the results discussed in this review, TG2 appears to be a promising target for containment and treatment of this formidable disease.

Keywords Chemoresistance · Metastasis · Invasion · Autophagy · NF-κB · FAK · PTEN

Introduction

Pancreatic ductal adenocarcinoma (PDA) is one of the most aggressive malignant diseases and fourth leading cause of cancer-related death world-wide (Jemal et al. 2006). The inability to diagnose the disease at an early stage (due to lack of symptoms), rapid metastasis and intrinsic resistance to currently available therapies are major reasons for the poor survival rates in these patients (median overall survival duration 4–6 months). Due to high intrinsic resistance to chemo- and radiation therapies, surgical resection remains the only curative hope for PDA patients. Unfortunately, the disease is so aggressive that only 10–20% of patients qualify for surgery at the time of initial diagnosis (Maheshwari and Moser 2005). These dismal figures clearly suggest the need for new therapeutic approaches for treatment of pancreatic cancer. In this respect, the identification of tumor-encoded genes whose expression contributes to the development of drug resistance and metastatic phenotypes may yield immediate clinical benefits. TG2 is one such protein whose expression has been found upregulated in several cancer cell types, selected for resistance to drugs or isolated from metastatic sites. Importantly, recent published data have provided strong experimental link between TG2 expression and increased invasion and drug resistance in cancer cells (for review see Verma and Mehta 2007a, b; Mehta et al. 2006). This chapter summarizes current knowledge on TG2 in context to cancer cells growth, survival and evaluates its potential as a therapeutic target.

TG2—a multifunctional protein

TG2 is structurally and functionally a complex protein with both intracellular and extracellular functions. In addition to catalyzing Ca^{2+}-dependent transamidation reactions (Lorand and Graham 2003; Griffin et al. 2002; Mehta 2005), it can bind and hydrolyze GTP/GDP with an affinity and a

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catalytic rate similar to the α subunit of large heterotrimeric G proteins and small Ras-type G proteins (Nakaoka et al. 1994; Mhaouty-Kodija 2004). In addition, TG2 can serve as a protein disulfide isomerase (Hasegawa et al. 2003) and a kinase (Mishra and Murphy 2004). As a scaffold protein, TG2 can induce FAK activation (Akimov et al. 2000; Verma et al. 2006) and its downstream signal transduction pathways such as PI3 K, Akt, and Ras/Erk and thus impact cell growth, survival and invasion functions (Guan 1997). Although predominantly a cytosolic protein, TG2 can be secreted outside the cell, where it crosslinks the extracellular matrix (ECM) proteins and renders the ECM resistant to mechanical and proteolytic insults (Aeschlimann and Thomazy 2000). TG2-mediated-crosslinking of ECM plays a role in the deposition and stabilization of the ECM, which in turn promotes cell attachment and spreading (Verderio et al. 2005). TG2 can translocate to the cell membrane where it binds tightly to both the cell-surface integrins and fibronectin and promote cell attachment, migration and invasion (Akimov et al. 2000; Akimov et al. 2001; Herman et al. 2006). Another enigmatic feature of TG2 is that it can function as a pro-apoptotic (Rodolfo et al. 2004*)* as well as an anti-apoptotic (Yamaguchi and Wang 2006) protein. Under physiological conditions, TG2 in the cytosolic compartment (where GTP concentration is approximately 100 μM (IC_{50} for GTP = 9 μM; Lai et al. 1998) and the free calcium level is around 100 nM [V_{max} for Ca^{2+} = 2 mM; Datta et al. 2006]) is enzymatically inactive. This cytosolic inactive fraction of TG2 may fulfill another function, that is to confer protection to cells from stress (reactive oxygen species, hypoxia, hormones, ultraviolet radiations, TNF and drugs, etc.). In this context it is worth mentioning the observation we made several years back that macrophages collected from inflammatory site accumulated large amounts of TG2 protein (Khera and Mehta 1989). In view of the results of recent studies, we believe that induction of TG2 in inflammatory macrophages may be related to their migratory functions and protection from cytotoxic mediators that they produce in response to infectious agents. Indeed, numerous recent reports have documented the direct role for TG2 in promoting migratory functions of normal cells. Thus, expression of TG2 in T lymphocytes was shown to play a role in their transmigration across the endothelial cells (Mohan et al. 2003). TGF β-induced expression of TG2 in retinal pigment epithelial cells has been linked to their increased migration on fibronectin-coated matrices (Priglinger et al. 2004). Retinoic acid-induced TG2 expression in neuroblastoma SH-SY5 cells augmented their migration and invasion functions (Joshi et al. 2006). These findings imply that TG2 expression can promote invasion and migration of a number of cell types, the feature that is important for metastasis of cancer cells to distant sites. Under extreme stressful conditions, Ca^{2+} homeostasis may be perturbed resulting in the activation of cytosolic TG2, crosslinking of intracellular proteins and apoptotic (Fesus et al. 1996) or necrotic cell death (Nicholas et al. 2003). Despite the variety of functions in which TG2 participates, knockout mice show no phenotype and are anatomically, developmentally, and reproductively normal (Laurenzi and Melino 2001; Nanda et al. 2001).

TG2 in cancer cells

A number of recent reports suggest that TG2 plays a role in the development of certain types of cancer. Multiple studies have shown that TG2 protein is upregulated in cancerous tissues such as PDA (Verma et al. 2006), breast carcinoma (Mehta et al. 2004), malignant melanoma (Fok et al. 2006), ovarian carcinoma (Satpathy et al. 2007) and glioblastoma (Yuan et al. 2006) to name a few. Second, a positive correlation between the chemoresistance and metastatic potential of certain cancers with expression levels has been demonstrated (Mehta 1994; Mehta et al. 2004, 2006; Fok et al. 2006; Herman et al. 2006; Verma et al. 2006; Mangala et al. 2007). Third, in certain tumors TG2 expression has been shown to exert anti-apoptotic effects on cells while siRNA downregulation of TG2 protein or treatment with TG2 inhibitors sensitizes these cells to apoptosis (Antonyak et al. 2001; Akar et al. 2007; Herman et al. 2006; Kim et al. 2006; Verma et al. 2006; Yuan et al. 2006). In an attempt to identify metastasis-associated proteins by proteomic analysis, Jiang et al. (2003) observed TG2 as one of the 11 proteins that were selectively amplified in metastatic human lung carcinoma. Similarly, comprehensive analysis of more than 30,000 genes by three different techniques, revealed TG2 as one of the most differentially expressed genes in pancreatic tumors (Iacobuzio-Donahue et al. 2003). Treatment of cancer cells with epidermal growth factor (EGF) induced the expression of TG2 and protected cells from doxorubicin-induced apoptosis (Antonyak et al. 2004). Contrary to this, there are also reports suggesting that the expression of TG2 is downregulated in certain types of cancer (Birckbichler et al. 2000; Jones et al. 2006). Xu et al. (2006) recently identified GPR56 as a protein that is downregulated in highly metastatic malignant melanoma cells and showed that TG2 was binding partner for GPR56 suggesting that TG2 can act as a tumor suppressing protein through its interaction with GPR56. These conflicting results suggest that the relevance of TG2 to cancer biology may depend upon the type of cancer, location of cancer, and possibly the stage of the cancer. Therefore, precise understanding of TG2 functions in context to cancer stage and type is important to implement TG2-based therapies.

TG2, integrins and extracellular matrix

Integrins are cell-surface proteins that serve as receptors for the ECM ligands (fibronectin, vitronectin, laminin, collagen etc.). Integrins can influence several aspects of cancer cell behavior, including motility, invasion, growth, and survival in response to their interactions with ECM ligands (Felding-Habermann 2003). In general, integrins represent low-affinity receptors (10^6–10^9 liters/mole) and can bind to their ECM ligands only when they exceed certain critical numbers in the form of focal contacts or hemidesmosomes. In response to certain stimuli, integrins can cluster and their combined weak affinities give rise to a spot on the cell surface (focal contact) that now has enough avidity to support stable interaction of cells with the ECM. Thus, under normal circumstances the interaction of integrins with their ECM ligands is tightly regulated to ensure controlled growth, survival and migration of cells. Interestingly, certain cellular proteins can selectively bind to integrins and promote their affinity for the ECM. The first such interaction was identified between integrin $\beta 3$ and the integrin-associated protein (IAP), which is also referred to as CD47 (Brown and Frazier 2001). The signaling cues transmitted by a specific integrin inside the cells can be modulated as a result of such interaction between integrin and the protein. In view of these observations, the finding that PDA cells express high basal levels of TG2 (Verma et al. 2006; Iacobuzio-Donahue et al. 2003) and that TG2 is associated in complex with β integrins (Akimov et al. 2000; Akimov and Belkin 2001; Herman et al. 2006; Fok et al. 2006), it is tempting to speculate that TG2 expression may regulate integrin-mediated signaling in cancer cells. Indeed, it is estimated that 10–40% of $\beta 1$ integrin on the cell surface could exist in 1:1 complex with TG2 (Zemskov et al. 2006). The interaction of TG2 with integrins can thus facilitate the adhesion and migration of normal as well as malignant cells on the ECM proteins (Mangala et al. 2007; Akimov and Belkin 2001). In line with these observations, Satpathy et al. (2007) recently reported that increased expression of TG2 in ovarian cancer cells enhanced their adhesion to fibronectin and promoted directional cell migration. Accordingly, ovarian cancer cells with knockdown TG2 showed diminished tumor dissemination on the peritoneal surface and mesentery in an intraperitoneal ovarian xenograft mouse model. Thus, aberrant expression of TG2 in cancer cells could confer cell survival and metastatic advantages by promoting integrin-mediated signaling.

TG2 and focal adhesion kinase (FAK)

FAK is a nonreceptor protein tyrosine kinase that plays a significant role in cell survival, migration and invasion (McLean et al. 2003). It is a key component of the signal transduction pathways triggered by integrins and is initiated at sites of integrin-mediated cell adhesion the so-called focal adhesion and carries out protein-protein interaction adaptor functions at sites of cell attachment to the ECM. FAK is activated by integrin clustering and transmits adhesion-dependent and growth factor-dependent signals into the cell interior to promote cell growth, cell survival and tumor invasion. Overexpression and/or increased activity of FAK have been frequently observed in a wide variety of human cancers at advanced stages. For example, increased levels of FAK were reported in 17 of 20 invasive tumors, and in 15 of 15 metastatic tumors of different origins but not in six normal tissue samples (Weiner et al. 1993). Similarly, increased levels of FAK protein were reported in 100% of colon and 88% of breast tumor samples, with FAK expression often being associated with advanced disease (Owens et al. 1995). Moreover, activation of FAK has been shown to enhance the adhesion and invasion of pancreatic cancer cells (Sawai et al. 2005). Given the important role of FAK in a large number of processes involved in tumorigenesis and metastasis, FAK can be a potential target in the development of anti-cancer drugs (McLean et al. 2003; Nimwegen and Water 2007). FAK itself can be regulated by a range of mechanisms, including tyrosine phosphorylation, serine/threonine phosphorylation and protein–protein interactions (Schlaepfer et al. 1999). For example, the association between TG2 and b1 integrin has been shown to promote the activation of FAK (Herman et al. 2006; Akimov and Belkin 2001). Importantly, our recent data suggested a direct role of TG2 in activation of FAK (autophosphorylation; pY^{397}) and its downstream PI3 K/Akt pathways in various cancer cell types, including pancreatic cancer (Verma et al. 2006). From these results, it is conceivable that TG2 expression contributes to the increased tumorigenic potential by constitutively activating FAK and its downstream pathways (Fig. 1).

TG2 and NF-κB activation

The transcription factor NF-κB has important roles in regulating genes involved in controlling cell growth, apoptosis, and metastatic functions. Constitutive activation of NF-κB has been observed in various cancers, and it has been posited to be implicated in drug resistance and metastasis (Karin et al. 2002). However, the molecular mechanisms that underlie constitutive activation of NF-κB remain elusive. From the observations that drug-resistant and metastatic cancer cells express high basal levels of TG2 and that TG2 expression contributes to the development of chemoresistance and metastatic phenotypes in

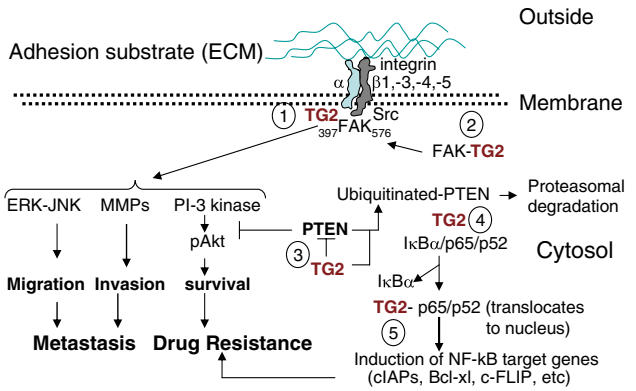

Fig. 1 TG2-medited activation of cell growth, cell survival and invasive functions. Aberrant expression of TG2 can activate FAK and its downstream signaling (Akt/ERK/MMP-2) pathways as a result of its association with *β* integrins. Association of TG2 with integrins can enhance their affinity for the ECM and promote integrin-mediated signaling (*1*). Alternatively, direct interaction between TG2 and FAK can induce autophosphorylation (pY397) of FAK protein, promote its association with beta1 integrin and induce outside-in signaling (*2*). TG2 can bind and promote ubiquitination and proteasomal degradation of the PTEN protein resulting in constitutive activation of Akt (*3*). Similarly, TG2 expression results in constitutive activation of NF-κB either by catalyzing polymerization of the inhibitory IκBα protein (*4*) or by binding to IκBα and preventing its interaction with the p65/p52 subunit of the NF-κB (*5*)

cancer cells, we hypothesized that TG2 expression could contribute to the constitutive activation of NF-κB. Indeed, expression of TG2 in various cancer cell types is associated with constitutive activation of NF-κB (Mann et al. 2006). NF-kB activity was high in Panc-28, A-375, MCF-7/DOX, and MDA231/cl.16 cell lines that express high basal levels of TG2. On the other hand, BxPc-3, WM-35, MCF-7, and MDA231/cl.9 showing low TG2 activity and expression showed low NF-kB activity. Forced expression of TG2 in the low-TG2-expressing BxPc-3 cells resulted in spontaneous activation of NF-κB. Conversely, knockdown of endogenous TG2 expression by siRNA in Panc-28 cells resulted in strong inhibition of NF-κB. Interestingly, in situ activation of TG2 transamidation activity by exposure to the calcium ionophore A23187 caused strong activation of NF-κB, and inhibition of transamidation activity by enzyme-specific inhibitors such as 5-(biotinamido)pentylamine and monodansylcadaverine attenuated NF-κB activation. Similarly, Kim et al. (2006) observed that cystamine (TG2 enzyme inhibitor) inhibited the NF-kB activation and reverses the doxorubicin resistance in breast cancer cell. Thus, TG2 expression and its cross-linking function are critical for NF-κB activation. Indeed, IκBα has shown to be a good substrate for TG2-catalyzed cross-linking reaction. The in vitro incubation of IκBα protein with purified TG2 and calcium effectively catalyzed the cross-linking of IκBα into high molecular weight polymers. Importantly, the polymeric form of IkBα had much lower binding affinity for the p65/p50 complex (Lee et al. 2004). We observed a similar posttranslational modification of IκBα in TG2-expressing cancer cells (Mann et al. 2006), suggesting that TG2-mediated posttranslational modification of IκBα could hamper its ability to associate with the p65/p50 complex, resulting in constitutive activation of NF-κB. The role of TG2 in NF-κB activation was further supported by the observation that transient transfection of a secretary alkaline phosphatase (SEAP) reporter construct in TG2-deficeint MCF-7 cells resulted in its expression (a 14-fold increase) only in response to treatment with TNF-α. This expression could be effectively blocked by dominant-negative IκBα but not by TG2 inhibitors. In contrast, high TG2-expressing Panc-28 cells showed a substantial increase (13-fold) in SEAP expression in absence of TNF treatment and this expression was significantly inhibited ($P < 0.002$) by TG2 inhibition. SEAP expression did not increase further in response to treatment with A23187 (1 mM for 24 h) and was not inhibited by dominant-negative IκBα (Mann et al. 2006). These results suggest that TG2-mediated activation of NF-κB is mediated via a novel pathway that is independent of IKK.

We also observed that TG2 could directly associate with p65/p50 complex in the cytoplasm and with p65 in the nucleus. We speculate that association of TG2 with the p65/p50 complex could interfere with the binding of IkBα to NF-κB complexes in the cytoplasm, resulting in its constitutive activation. Under certain conditions, TG2 can also serve as a kinase, and its serine-threonine kinase activity can phosphorylate histones and p53. Because p65 undergoes phosphorylation by various kinases at Ser536, it is likely that p65 serves as substrate for TG2 kinase activity. In addition, it is also possible that TG2, being a bulky protein (80 kDa), in complex with p65 could cause stearic hindrance or conformational changes and allow preferential binding of NF-κB (p50/p65) complex only to high-affinity or selective promoters, resulting in differential transcriptional regulation and expression of target proteins involved in drug resistance and metastasis. All these possibilities remain to be investigated. Nevertheless, aberrant expression of TG2 results in constitutive activation of NF-κB and transduction of downstream cell survival genes.

Downregulation of TG2 promotes drug sensitivity

We made an important observation in siRNA-transfected PDA cells; downregulation of TG2 by siRNA was associated with autophagic death (type II programmed cell death) (Akar et al. 2007). Thus, transfection of Panc-28 or Capan-1 cells with TG2-specific siRNA (but not control-siRNA) induced massive accumulation of autophagic vacuoles as revealed by phase-contrast microscopy. The vacuoles were

highly acidic in nature as determined by acridine orange staining and were accompanied by accumulation of microtubule-associated light chain (LC3) protein, the hallmarks of cells undergoing autophagy (Tanida et al. 2005; Klionsky 2007). In control siRNA-treated cells none of these changes were evident. We further analyzed the autophagy in TG2 downregulated Panc-28 by transmission electron microscopy (TEM). The TEM data revealed the formation of autophagosomes containing cellular organelles in TG2 siRNA-transfected Panc-28 cells but not in control siRNA-treated cells. In addition to the appearance of autophagic vesicles the TEM images also revealed the merging of autophagic vesicles with lysosomes and mitochondria and cellular organelles and lysed cellular contents within the autophagosomes (Akar et al. 2007), suggesting lysosomal-mediated degradation of cellular organelles. Although the role of autophagy in cell death has been controversial, recent reports have started to note that autophagy can drive cells into cell death (Levine 2007). Persistent activation of autophagy can lead to programmed cell death (Yu et al. 2004). The autophagy regulator beclin 1 (*BECN1*) is a haploinsufficient tumor-suppressor gene that induces autophagy when overexpressed further supports that autophagy under certain conditions can lead to cell death. It is tempting to speculate that inhibition of TG2 expression deprives the cells of survival signaling pathways and thus could lead to autophagic death in cancer cells. Indeed, inhibition of endogenous TG2 by siRNA rendered PDA cells sensitive to gemcitabine (Verma et al. 2006). Similarly, knockdown of TG2 increased the sensitivity of ovarian and breast cancer cells to drug-induced cell death (Herman et al. 2006). Treatment of glioblastoma cells in culture with the competitive TG2 inhibitor, monodansylcadarevine (MDC) or with the selective small molecule-irreversible TG2 inhibitor, KCA075, or its analog KCC009 showed an increased incidence of tumor cell apoptosis (Yuan et al. 2006). In addition, KCC009 treatment in mice harboring orthotopic glioblastomas sensitized the tumors to *N,N′*-bis(2-chloroethyl)-*N*-nitrosourea chemotherapy, as measured by reduced bioluminescence, increased apoptosis and prolonged survival. Our systemic siRNA approach offers an alternative option for therapeutic targeting of TG2.

TG2 as a therapeutic target

To determine in vivo significance of elevated TG2 expression in growth and survival of PDA cells, we selected two stable TG2-shRNA transfected clones (Panc-28/cl.12 and Panc-28/cl.10) which expressed 70–80% less TG2 than the parental Panc-28 cells (Verma et al. 2008a). Xenografts were grown subcutaneously in nude mice using TG2 knockdown (Panc-28/cl.10) and TG2-expressing (Panc-28/empty vector; control) PDA cells. Panc-28/cl.10 xenografts showed significantly retarded growth compared with the Panc-28/empty vector controls (Verma et al. 2008a). The tumor growth was followed for 5 weeks and mice were sacrificed. There was a 60–80% reduction in tumor volumes of TG2 knockdown Panc-28/cl.10 xenografts compared to empty vector-Panc28-induced xenografts ($P = 0.017$). Western blot analysis of xenografts at the end of fifth week revealed that Panc-28/cl.10 tumors continue to express low TG2 compared to the control tumors. Moreover, Panc-28/empty vector tumors showed strong constitutive NF-κB activity, whereas TG2 knockout Panc28/cl.10 tumors lacked this activity.

Next, we tested the therapeutic ability of TG2 siRNA for treating PDA tumors. Several recent reports have documented that liposomes composed of neutral lipid dioleyl phosphatidylcholine (DOPC), can effectively deliver gene-specific siRNA in vivo. Thus, DOPC-siRNA is 10- and 30-fold more efficacious in delivering siRNA to the tissues than the cationic liposomes (DOTAP) or naked siRNA, respectively. DOPC-mediated delivery of siRNA resulted in significant downregulation of the target protein (up to 95%) and inhibited the growth of ovarian carcinoma (Landen et al. 2005; Halder et al. 2006; Merritt et al. 2008) and colorectal carcinoma (Gray et al. 2008) in mouse models. Therefore, we used DOPC liposomes to deliver TG2 siRNA for treating PDA tumors growing orthotopically in nude mouse. Panc-28 cells (1×10^6; stably transduced with the firefly *luciferase* gene) were injected into the pancreases of male athymic *nu/nu* mice and tumor growth was monitored weekly by measuring bioluminescence using a cryogenically cooled IVIS imaging system coupled with a data acquisition computer running Living-Image software (Xenogen Corp., Alameda, CA, USA). A digital grayscale image of each animal was acquired followed by acquisition and overlay of a pseudocolor image of the spatial distribution of photons emerging from active luciferase within the tumor. The signal intensity was quantified as the sum of all detected photons within the region of interest per second per square centimeter per steridian. Luciferase bioluminescence data derived from weekly IVIS images revealed that the tumor growth in mice given TG2-siRNA-DOPC in combination with gemcitabine was significantly retarded ($P = 0.019$) when compared with that in the mice given control siRNA-DOPC (Verma et al. 2008b; Fig. 2a). Treatment with either TG2 siRNA-DOPC or gemcitabine alone resulted in about 50% reduction in the final tumor volumes (Fig. 2b). Thus, the combination of TG2 siRNA-DOPC and gemcitabine had superior efficacy compared with TG2 siRNA-DOPC or gemcitabine alone. Mice tolerated siRNA-DOPC treatment extremely well as there was no noticeable change in the

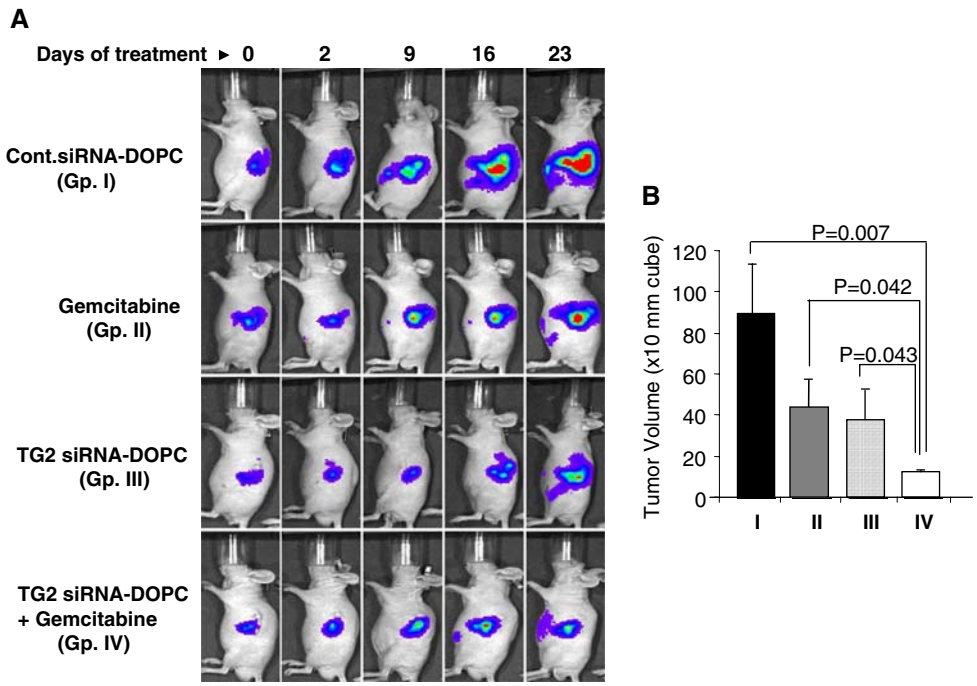

Fig. 2 Downregulation of TG2 inhibits growth of orthotopically growing PDA tumors in mice. **a** IVIS images of representative mice in each treatment group taken at the indicated days of treatment. Mice were imaged in the ventral view using the LivingImage Software program. **b** Final tumor volumes in mice at the end of treatment. Treatment with TG2 siRNA-DOPC alone (*group III*) or gemcitabine alone (*group II*) resulted in 50-60% reduction in tumor growth when compared with treatment with control siRNA-DOPC (*group I*). Treatment with the combination of TG2 siRNA-DOPC and gemcitabine (*group IV*) resulted in a much superior response when compared with treatment with TG2 siRNA-DOPC or gemcitabine alone ($P < 0.05$)

body weight or eating habits of mice. The anti-tumor activity was related to decreased proliferation, angiogenesis and increased tumor cell apoptosis in vivo. Taken together, these results suggest that TG2 overexpression is an adverse prognostic factor in PDA cancer and represents an attractive therapeutic target.

Conclusions

The observation of elevated TG2 expression in various cancer cell types have raised great interest in understanding the contribution of this protein to the development of drug resistance and metastatic phenotype. Acquired or intrinsic resistance to a wide variety of drugs and metastatic spread of cancer cells to distant sites (metasitasis) pose major clinical challenge in successful treatment of cancer. Literally, all the more than 6.5 million annual cancer-related death worldwide can be said to have occurred because the chemotherapy in these patients failed and/or tumor cells metastasized to distant sites. The findings that TG2 expression results in a constitutive activation of cell survival and invasive signaling pathways, imply that TG2 can inhibit apoptosis/autophagy in cancer cells and thus enable tumor cells not only to display resistance against anticancer therapies but also to survive successfully in the stressful environments of distant tissues (metastasis). On the basis of these results, we conclude that TG2 represents an attractive therapeutic target for overcoming drug resistance and inhibiting metastasis of cancer cells. Indeed, our initial experiments using highly aggressive orthotopically growing pancreatic tumor cells serve as a proof-of-concept in support of this contention and demonstrated that downregulation of TG2 could render cancer cells sensitive to drugs and inhibit their metastasis.

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