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Tissue transglutaminase in tumour progression: friend or foe?

Review Article

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Summary. Basic biological processes in which tissue transglutaminase (TG2, tTG) is thought to be important including apoptosis, cell adhesion and migration, ECM homeostasis and angiogenesis are key stages in the multistage tumour progression cascade. Studies undertaken with primary tumours and experimental models suggest that TG2 expression and activity in the tumour body and surrounding matrix generally decreases with tumour progression, favouring matrix destabilisation, but supporting angiogenesis and tumour invasion. In contrast, in the secondary metastatic tumour TG2 is often highly expressed whereby its potential roles in cell survival both at the intra- and extracellular level become important. In the following review the underlying molecular basis for the selection of these different phenotypes in tumour types and the anomaly for the requirement of TG2 is discussed in relation to the complex events of tumour progression.

Keywords: Enzyme – Tissue transglutaminase – Tumour growth – Angiogenesis – Extracellular matrix – Review

Abbreviations: bFGF, basic fibroblast growth factor; EC, endothelial cell; ECM, extracellular matrix; EGF, endothelial growth factor; FAK, focal adhesion kinase; LTBP, latent transforming growth factor-β binding protein; MMP, matrix metalloproteinase; NF-kappa *B*, nuclear factor-kappa *B*; NO, nitric oxide; TG2, tissue transglutaminase; TGF-β, transforming growth factor β; TIMP, tissue inhibitor of matrix metalloproteinases; uPA, urokinase plasminogen activator; uPARAP, urokinase plasminogen activator receptor associated protein; VEGF, vascular endothelial growth factor

Introduction

Cancer remains one of the leading causes of mortality worldwide and its incidence will continue to rise in the next 20 years despite our increased understanding of the basic biological processes that become disturbed in tumourigenesis and metastasis. The advent of improved treatment regimes in surgery, radiotherapy, chemotherapy and more recently gene therapy are either restricted in marginal improvement of metastasis or are limited to specific types of tumours. Consequently there is a pressing need to complement reactive post-diagnosis tools with proactive research on the cellular mechanism of tumour growth and spread, if step changes in the treatment against cancer are to be realised.

In vitro transformation is itself a multi-step process. Pathological analyses of organ sites often reveal lesions that seem to represent intermediate steps in a process through which normal cells evolve progressively via a series of pre-malignant states into invasive cancers. The transition from the benign to the malignant phenotype is marked by altered expression of a range of gene products. Rodent cells require a minimum of two introduced changes in their genetic make-up before acquiring tumorigenic competence (Hahn et al., 1999). Once the malignant phenotype is established resulting in the primary tumour, tumour progression is driven by a series of events involving a number of differing phenotypes and ultimately resulting in establishment of the secondary tumour (Foulds, 1954). Decreased cell–cell contact enables malignant cell disengagement from the primary tumour site, which in synergy with extracellular matrix destabilisation allows for their migration. Degradation of the basal membrane facilitates invasion into the vasculature or lymphatic system, which is followed by rolling and arrest of the malignant cells at a distant site. Tumour cells can then attach to the endothelial cell (EC) lining, often associated with a specific tissue type depending on the primary tumour. Invasion into the sur-

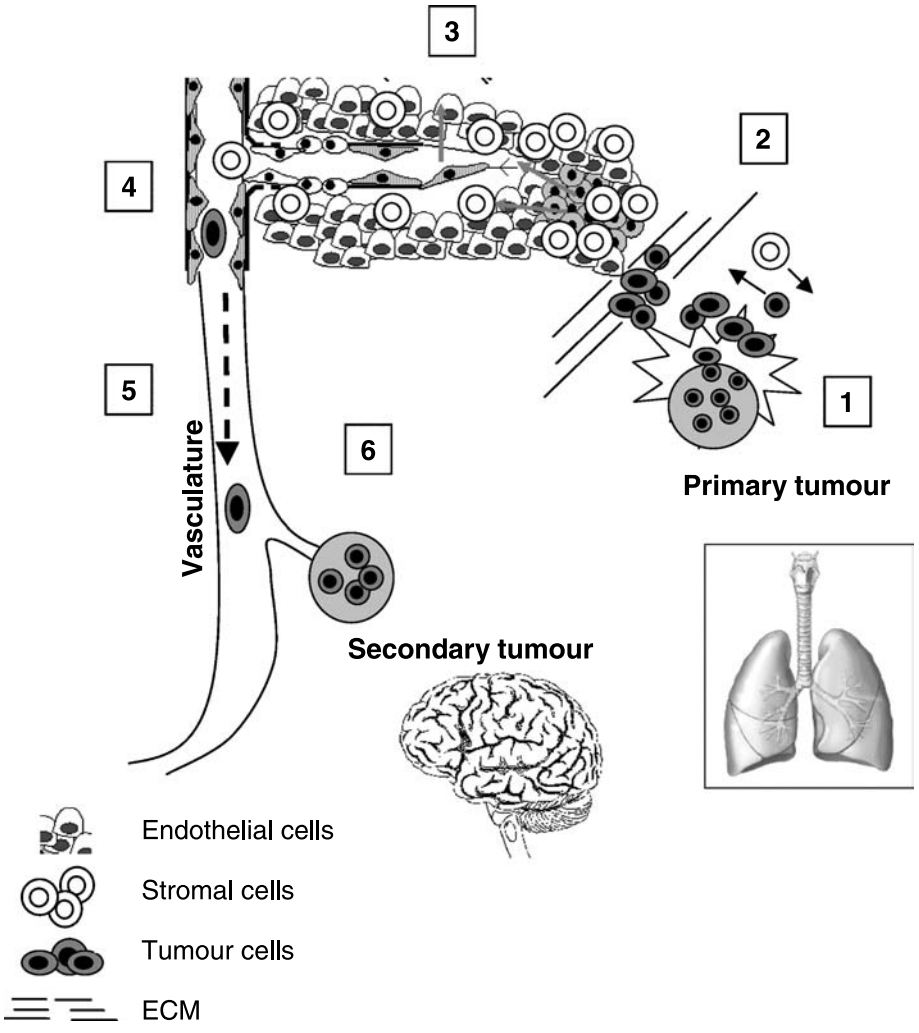

Fig. 1. The tumour growth and metastatic cascade. *1*. Transformed cells at an organ site acquire tumorigenic competency through alterations in their gene expression. *2*. Tumour cells disengage from the primary tumour site and migrate outwards towards the surrounding stroma, whilst stromal cells migrate in the opposite direction towards the tumour site. In this bidirectional process tumour cells recruit host or secrete themselves proteases to breakdown stromal ECM. *3*. Once the tumour expands to 1–2 mm in size, VEGF and ang-1 signal for EC mobilisation leading to capillary sprout formation. *4*. Tumour cells degrade the basement membrane and enter the vascular system. *5*. Tumour cells roll within the lumen of the capillary prior to arrest at a distant organ site. *6*. Degradation of the basement membrane facilitates extravasation of tumour cells into secondary tumour site

rounding tissue then requires passage through the EC junctions and degradation of the basal membrane, a process referred to as extravasation (Fig. 1).

Malignancy is a state that emerges from the tumour-host microenvironment in which the host participates in the induction, selection and expansion of the neoplastic cells (Park et al., 2000). Neoplastic cells continuously stimulate host stromal and vascular cells to assist them in conducting physiological invasion. Motility and invasion is a bi-directional process, as within the same tumour microenvironment capillary sprouts migrate and invade towards the tumour mass, while tumour cells migrate outwards in the opposite direction (Liotta and Kohn, 2001). The tumour-host interdependence can be best manifested in that most of the enzymes and inhibitors complexed at the invasion front are contributed by the host cells, not by the invading tumour cells (Nakahara et al., 1997). Tumour cells produce angiogenic factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), which stimulate stromal vascular cells causing vascular permeability, endothelial proliferation and invasion. On the other hand fibroblast and ECs secrete latent enzymes, among which matrix metalloproteinases (MMPs) and urokinase plasminogen activator (uPA), which dock on the surface of the carcinoma invadopodia where they become activated, thus degrading the ECM (Liotta and Kohn, 2001). Conversely, ECM degradation releases bound growth factors such as transforming growth factor-β (TGF-β) and endothelial growth factor (EGF), which in turn bind to their cognate receptors on the malignant cells. Ongoing

extracellular matrix (ECM) degradation also exposes cryptic RGD sites, which are recognized by integrins.

Basic biological processes involving both stromal and malignant cells such as apoptosis, adhesion and migration, ECM homeostasis and angiogenesis are key stages in this multistage tumour progression cascade and as such have become fertile areas in the hunt for rationally selected anticancer agents.

Tissue transglutaminase (TG2) belongs to a family of mammalian enzymes that post-translationally modify proteins in a calcium-dependent manner leading to the formation of covalent ε(γ-glutamyl)lysine linkages (Griffin et al., 2002). Nine genes have so far been identified, of which six proteins have been characterised. Five of these show Ca^{2+}-dependent transamidating activity (Griffin et al., 2002). The other protein, Band 4.2, is a red cell membrane associated protein and is catalytically inactive. TG2 is localised inside the cell in free cytosolic, mitochondrial, and nuclear forms, in the extracellular environment and in association with the cell surface (Griffin et al., 2002). The presence of TG2 within the different cellular compartments is thought to serve distinct physiological functions. Under normal physiological conditions transamidating activity is tightly regulated in the intracellular environment by the presence of low Ca^{2+} and the inhibitory effects of GTP/GDP (Smethurst and Griffin, 1996). However, under conditions of cell stress/trauma, following disturbance or loss of Ca^{2+} homeostasis, crosslinking of intracellular proteins may occur as observed during the onset of apoptosis (Fesus et al., 1989), or necrosis (Nicholas et al., 2003).

TG2 is also translocated to the cell surface by an unknown secretory mechanism which appears to require an intact N-terminal β-sandwich domain and the active site conformation of the enzyme (Gaudry et al., 1999). At the cell surface and in the extracellular space TG2 is reported to play an important role in both cell adhesion and matrix stabilisation. Various important functions have therefore been ascribed to the enzyme in both the intra- and extracellular environment including its role in matrix stabilisation, cell adhesion and migration (Griffin et al., 2002) and in cell death and survival (Fesus and Szondy, 2005). This review will try to demonstrate how some of these functions are essential to the concerted events of tumour progression.

Importance of TG2 in the different stages of tumour progression and metastasis

TG2 and malignant cell proliferation and apoptosis

Insensitivity to growth signals and evasion of apoptosis are prerequisites for malignant cells to proliferate and infiltrate the surrounding host tissue (Hanahan and Weinberg, 2000). A few lines of evidence suggest that TG2 might be involved in the relay of anti-growth signals during tumour growth (Birckbichler and Patterson, 1978). Stable transfection of a malignant hamster fibrosarcoma (MetB) with active and inactive TG2 leads to delayed progression of cells from S-phase to G2/M in both variants (Mian et al., 1995). Negative regulators of cell cycle are inactivated during cancer development and the concomitant decrease in TG2 expression in the developed tumours is possibly a manifestation of such a feedback mechanism (Mian et al., 1995). In a recent report, TG2 was also shown to induce constitutive expression of NF-kappa *B*, a regulator of cell growth and apoptosis, independent of NF-kappa *B* kinase (Mann et al., 2000). Interestingly, the cytoplasmic domains of the mouse and rat cancer adhesion molecule (C-CAM-L) are substrates of TG2, and once covalently modified, form stable dimers that might elicit a negative growth signal (Hunter et al., 1998).

Several reports highlight the involvement of intracellular TG2 both as a pro- and anti-apoptotic factor (Nicholas et al., 2003; Mehta, 1994; Piacentini et al., 1996). TG2 mRNA was detected in 75% of breast carcinomas with high apoptotic index and only in 29% of those with low indices (Grigoriev et al., 2001). Apoptotic cells often express high levels of TG2, thought to prime cells for programmed cell death. TG2 mediated tumour cell death can be brought about either in a classical apoptotic manner, or through a distinct TG2-mediated cell death mechanism. Induction of TG2 by retinoic acid in adenocarcinoma and neuroblastoma cell lines sensitise cells to apoptosis due to mitochondrial hyperpolarisation (Melino et al., 1994; Piacentini et al., 2002). However other data indicate chemical induction of TG2 in hamster fibrosarcoma cell lines leads to unaltered rates of classical apoptosis but to an increase in crosslinked detergent insoluble apoptotic envelopes (Johnson et al., 1998). In this study, anti-apoptotic bcl2 expression by the fibrosarcoma cells did not rescue malignant cells from cell death, suggesting a distinct TG2-mediating cell death mechanism is in place (Johnson et al., 1994). It is believed that TG2 may play a role downstream of the apoptotic cascade, as part of a 'fail safe' mechanism separate from the cell-death commitment machinery that ensures protection against excessive inflammation mediated by necrosis following loss of Ca^{2+} homeostasis (Nicholas et al., 2003).

Inside the cell TG2 has been reported to mediate pro-apoptotic signals through transamidation of key proteins such as pRB (Oliverio et al., 1997). Following cell insult, loss of membrane integrity allows Ca^{2+} influx, which activates TG2 within the cell and facilitates intracellular

protein cross-linking. Extensive cross-linking prevents intracellular components from leaking into the extracellular space, where they could induce an anti-inflammatory response (Nicholas et al., 2003; Fesus and Szondy, 2005). Conversely, at the cell surface TG2 plays an anti-apoptotic role. TG2 association with integrins induces activation of anti-apoptotic protein Bcl-2, which could in turn activate focal adhesion kinase (FAK) signal transduction pathways such as PI3K/Akt, and Ras/Erk (Guan, 1997). A comparable survival signaling pathway can also be induced when TG2 is deposited into the matrix and associated with FN through an RGD independent pathway involving heparan sulphate proteoglycan (HSPG) receptors of which Syndecan 4 and 2 are the likely contenders (Verderio et al., 2003).

Cell surface TG2 and tumour cell adhesion

Cell adhesion plays a pivotal role in the protection of malignant cells against anoikis in an outside-in signal transduction mechanism. However, during primary tumour growth, increased cell adhesiveness, could prove unfavourable to the tumour progression cascade, as loss of malignant cell to cell contact allows for cells to move freely out of the tumour mass. A plethora of evidence suggests that TG2 overexpression is responsible for increased cell attachment and spreading, whereas reduced expression of TG2 leads to impairment of these functions (Jones et al., 1997; Verderio et al., 1998). As mentioned earlier, a putative role for TG2 as an integrin-binding adhesion coreceptor for fibronectin (Akimov et al., 2000), and more recently a novel TG2-mediated, RGD-independent cell adhesion mechanism involving HSPGs that rescues cells from anoikis have been proposed (Verderio et al., 2003). As a consequence one might expect TG2 expression to be reduced but not absent in the early stages of tumour progression where cell migration is essential to tumour invasion. However, whether cell surface TG2 favours or opposes cell migration also depends on the ECM composition. The proteolytic degradation of glioma and fibrosarcoma cell-surface TG2 following overexpression of MT1-MMP suppresses cell adhesion and locomotion of the tumour cells on fibronectin (FN), yet stimulates cell motility on collagen matrices (Belkin et al., 2001). Interestingly, the crosslinking of collagen by TG2 results in increased cell adhesion and spreading on the modified collagen (Chau et al., 2005).

Activated H-Ras and Raf-1 oncogenes decrease TG2 biosynthesis, surface expression and association with β1-integrins in transformed NIH-3T3 fibroblasts, thus impairing TG2-mediated adhesion (Akimov and Belkin, 2001). TG2-siRNA studies on HeLa cells indicate that inhibition of adhesion and motility is due to reduced phosphorylation of Akt and NOS (Mehta et al., 2004). The observation that the tumour suppressor cell adhesion molecule C-CAM is a substrate for TG2, also raises the possibility that TG2 may exert a negative effect on tumour growth via covalent modification of this molecule (Hunter et al., 1998). Neoplasms are not only found to downregulate intrinsic TG2 expression, but also express MT1-MMP and activate soluble MMP2 which can cleave cell surface associated TG2 of neighbouring host cells (Belkin et al., 2004). The resulting loss of host cell-matrix interaction may then serve to retard the host response.

TG2 in tumour-host ECM homeostasis

The extracellular matrix is a complex structural entity that provides stromal and tumour cells with mechanical support for adhesion and migration. Assembly and degradation of the ECM is a dynamic process balanced by protein component synthesis, under the regulation of TGF-β, and degradation, under the regulation of proteases (MMPs) and their tissue inhibitors (TIMPS). TGF-β1 stimulates the expression of collagen and FN, whilst facilitating their incorporation into the ECM. ECM regulation controls both host EC mobilisation during tumour angiogenesis, and malignant cell migration during invasion, intravasation and extravasation. As stated earlier, under normal physiological conditions the majority of TG2 activity in the cell cytosol is predicted to remain latent due to tight regulation by the presence of low Ca^{2+} and high GDP/GTP. The extracellular environment, however, provides a high concentration of Ca^{2+} and low concentration of nucleotides necessary for the activation of TG2. Once externalised from the cell TG2 has the capacity, if present in high enough concentrations, to induce qualitative changes to ECM proteins. It is difficult to predict how long TG2 is active for in the extracellular environment since it is a thiol dependent enzyme subject to oxidation, whilst its tight binding to FN may exert limitations on its crosslinking ability (Barsigian et al., 1991).

Under pathological conditions, increased presence of the enzyme in the ECM has been demonstrated both *in vitro* and in vivo to give rise to increased deposition and accumulation of ECM proteins (Jones et al., 2006; Johnson et al., 1999). This accumulation is facilitated by TG2 either via direct crosslinking, that confers resistance to proteolytic degradation and delays their turnover, or by activation of matrix bound TGF-β1. In the latter

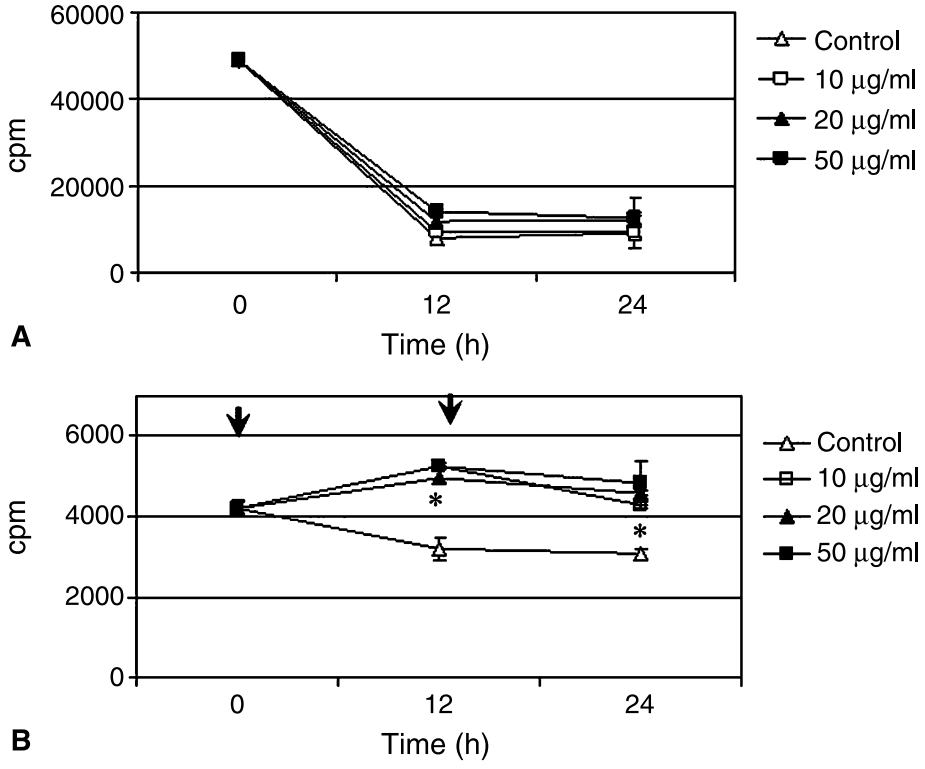

Fig. 2. Increased extracellular TG2 leads to a reduction in matrix turnover in human dermal fibroblasts. C378 human dermal fibroblasts were seeded at 2×10^5 cells/well in 24-well plates and allowed to grow overnight prior to labeling with 5 μCi/ml [2,3-^{3}H]-proline in standard cell growth medium for 48 h. Following washing in PBS, cells were administered with two doses of 10, 20, or 50 μg/ml active (50 U/ml) guinea pig liver TG2 in fresh label-free media at 0 and 12 h (arrows). Following removal of cells by EDTA, the ECM components were extracted sequentially in proteinase K and SDS. Scintillation counts of the radiolabel in the media and ECM fractions were taken at 0, 12, and 24 hours of culture growth. **A** Cell growth media fraction; **B** ECM fraction. Results represent mean values SD from two independent experiments, each undertaken in duplicate; * indicates significantly different from the untreated control ($p < 0.05$)

case it is thought that TG2 crosslinking can regulate the deposition/circulation balance of latent TGF-β precursor into/from the ECM, hence controlling de novo ECM protein synthesis (Verderio et al., 1999). Since many extracellular proteins are known to serve as substrates for TG2 (Griffin et al., 2002) and given the resistance of the ε-(γ-glutamyl)lysine isopeptide bond against degradation by MMPs, which are often docked on the surface of the carcinoma invadopodia it is believed that TG2 contributes to the low turnover (Barsigian et al., 1991; Jones et al., 2006) of the ECM and may prevent or delay remodelling of basement membranes (Fig. 2). A stable ECM is intrinsically anti-angiogenic and inhibitory towards malignant cell proliferation and migration because it is more resistant to protease digestion and mechanical disruption by the expanding tumour mass. Hence, the formation of a stable ECM may be an effective barrier to growth and metastasis of tumours by restricting infiltration by tumour cells and growth of new blood vessels (Haroon et al., 1999b).

Work undertaken in our laboratory suggests that TG2 activity and expression steadily decrease as the primary tumour expands in size (Fig. 3). Although still controversial, the majority of studies undertaken with primary tumours and experimental models suggest that reduced TG2 expression and activity in the tumour body and surrounding matrix favours primary tumour progression (Barnes et al., 1985; Hand et al., 1988; Jones et al., 2006; Mangala et al., 2005; Xu et al., 2006). This decrease might serve to destabilise the matrix facilitating both tumour spread and angiogenesis (Ingber, 2002; Jones et al., 2006). Stable expression of the human TG2 cDNA in a hamster fibrosarcoma and in a neuroblastoma cell line led to reduced incidence of primary tumour growth suggesting that reduction of TG2 expression and activity are causal rather an epiphenomenon of tumour growth (Johnson et al., 1994; Melino et al., 1994). Most of the enzyme expressed in the tumour-host microenvironment might be expected to be contributed by stromal cells probably as a result of the host's natural defense against tumour formation. Tumours are known to elicit a wound healing response from the host tissues, resulting in formation of granulation tissue at the advancing margins

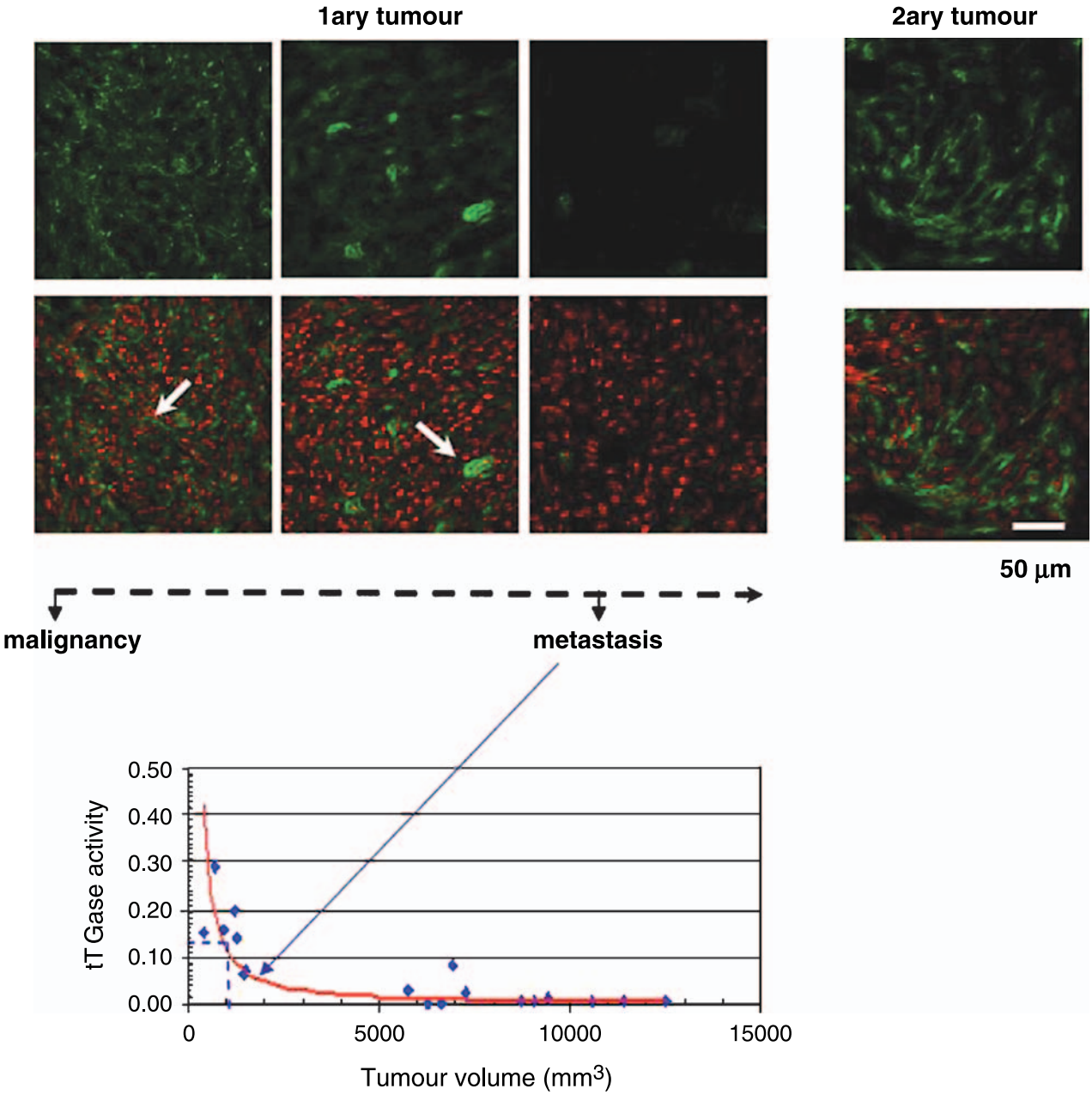

Fig. 3. Transglutaminase activity and expression at the different stages of tumour progression and metastasis. **A** Immunofluorescent analysis of TG2 in a highly malignant P8 rat osteosarcoma analysed at the different stages of tumour growth. **B** Measurement of transglutaminase activity in primary tumour homogenates by incorporation of [^{14}C]-putrescine into N,N′-dimethylcasein. Tranglutaminase activity is reduced as the tumour mass expands and appears to be lost in the mature tumour body. Conversely the secondary tumour exhibits increased transglutaminase activity

(Dvorak, 1986). Immunohistochemical analysis of human breast tumour tissue indicated that only 15% of the human breast carcinomas screened stained positive for TG2 antigen as opposed to a 50% of the stromal tissue (Grigoriev et al., 2001). In a separate study, similar analysis of intraductal breast cancer tissue indicated that TG2 antigen is higher at the boundary between stromal and tumour tissue (Hettasch et al., 1996). Host response mechanisms include induction of cytokines that promote wound healing, stimulation of the immune system to resist foreign invasion, upregulation of tumour suppressor genes, and more importantly ECM accumulation and stabilization. The role of TG2 in wound healing is now well documented (Telci and Griffin, 2006). Importantly it has been demonstrated in vitro that transglutaminases can accelerate collagen self-assembly whilst improving the mechanical properties

of collagen matrices, via cross-linking (Jones et al., 2006). Following the observation of increased TG2 expression in the tissue surrounding the tumour it has been hypothesised that TG2 participates in the body's natural defense against tumour formation (Hettasch et al., 1996). Indeed, challenge of C57/BL6 TG2 transgenic mice with B16-F1 melanoma cells led to increased tumour growth rates and reduced survival within the TG2 knock-out population when compared to their wild type counterparts (Jones et al., 2006). Immunohistochemical and western blotting studies confirmed the lack of TG2 antigen within the tumour body, as reported previously (Barnes et al., 1985; Hand et al., 1988). The presence of increased TG2 antigen at the boundary between the tumour body and the stromal tissue in TG2 wild-type mice suggested that the enzyme is possibly stabilising the ECM at the tumour-host interface, hence sealing off the tumour by inducing fibrosis. This is in keeping with a recent report suggesting that TG2 is expressed as a host response to tumour invasion and inhibits tumour growth (Haroon et al., 1999b).

A viable intervention strategy in containing the expanding tumour mass expansion might lie in mimicking the host response mechanism of fibrosis. On the back of this insight, a number of research groups have to date attempted to induce qualitative changes to the ECM with a view to limiting tumour cell invasion (Jones et al., 2006; Haroon et al., 1999b; Mangala et al., 2005). Exogenous TG2 administration has been shown to inhibit invasion of MDA-MB-231 breast cancer cells through matrigel in a dose dependent manner (Mangala et al., 2005). In a more recent study, mice lacking urokinase plasminogen activator receptor associated protein (uPARAP) which mediates intracellular collagen degradation, contained tumour progression, unlike their wild type counterparts (LeBrasseur, 2005). This study confirms the importance of collagen accumulation within the tumour as a barrier to tumour invasion. In another study, rather than modulating the capacity of stromal cells to digest collagen either intracellularly (lysosomes) or extracellularly (MMPs), TG2-induced overloading of the tumour ECM with collagen provided an effective barrier to tumor progression. Intratumour injection of CT26 mouse colon carcinoma tumours with active TG2 was also shown to inhibit early tumour growth (Jones et al., 2006). In this study increased collagen deposition and crosslink formation were indicative of a qualitative change in ECM balance. Similarly, in a rat dorsal window flap model, topical application of TG2 post-surgery was also found to inhibit both tumour growth and growth of new vasculatures when a solid piece of the rat mammary adenocarcinoma R3230Ac tumour was added to the wound (Haroon et al., 1999b). Tumour growth delay was accompanied by the onset of fibrosis as demonstrated by increased collagen deposition, suggesting hindered malignant and EC motility. However, in both studies tumour growth rates were comparable in control and treated tumours several days after treatment suggesting that a compensation mechanism was in place. This is in keeping with earlier studies that indicate stable transfection of a malignant hamster fibrosarcoma with TG2 leads to reduced primary tumour incidence yet parallel growth rates in vitro and in vivo once the tumour has matured (Johnson et al., 1998). Interestingly, when applied exogenously and at non-physiological levels on the human colon carcinoma cell line LS174T, transglutaminase was shown to inhibit tumour cell spreading (Zirvi et al., 1993).

Tissue transglutaminase in tumour angiogenesis

Angiogenesis is a prerequisite for neoplastic cells to grow into primary tumours and metastasise since solid tumours cannot expand in size beyond 1–2 mm in diameter in the absence of new independent blood supply (Folkman, 1990). New vasculatures also present new avenues for tumour escape into the circulation. Therefore, inhibition of angiogenesis, which involves inhibition of EC migration and growth, can translate into tumour growth inhibition by starving tumours of their blood supply. Angiogenesis requires proliferation and migration of ECs as well as their interaction with subendothelially located smooth muscle cells, fibroblasts and pericytes, the basement membrane and the ECM to form a complex three dimensional structure that contains endothelial monolayers surrounding the lumen of the vessel. This process is initiated under the control of hypoxia inducible factor in response to vascular endothelial growth factor. Release of VEGF induces vasodilation via endothelial nitric oxide (NO) production and its endothelial permeabilising effect, whilst promoting EC proliferation (Ziche et al., 1997). Once the EC has been activated, it migrates to the site of vascularisation, following degradation of the basement membrane by MMPs secreted in a latent form by ECs, fibroblasts and epithelial cells. Plasminogen activators u-PA and t-PA convert plasminogen to plasmin, which, apart from degrading basement membrane proteins such as FN and activating certain MMPs, mobilises bFGF from the ECM pool to up-regulate EC proliferation, motility and proteinase activity. Integrins ($\alpha v\beta 3$ and $\alpha v\beta 5$) have also been shown to be pivotal in endothelial survival during angiogenesis, as they rescue malignant cells from anoikis. The angiogenic process is regulated by growth factors, proteases, the

expression of cell surface receptors and the ECM. Whilst some of the proposed functions of TG2 such as cell adhesion, migration and ECM stabilization, are similar to the critical steps involved in angiogenesis, the role of TG2 in this process is poorly understood.

Expression of TG2 is relatively high in ECs (Korner et al., 1989). An increase in TG2 expression during dermal wound healing in rats was first established by Bowness and colleagues (1988). This increased expression of TG2 found in ECs and macrophages invading the fibrin clot results in the formation of transglutaminase-mediated cross-linking in both fibrin and the new granulation tissue during the formation of a provisional matrix (Haroon et al., 1999a). This finding is consistent with the observation that HUVEC are a rich source of TG2, the synthesis of which is up-regulated by thrombin (Auld et al., 2001). In the ECs, the crosslinking of ECM proteins by TG2 is thought to play an important physiological role in the stabilisation of the basement membrane (Martinez et al., 1994). Surprisingly, TG2 knockout mice do not present vascular abnormalities (De Laurenzi and Melino, 2001). Gene array analysis of differential gene expression during human capillary morphogenesis in 3-D collagen matrices has classified TG2 among the genes which are controlled during this process (Bell et al., 2001). In that study, TG2 was found to be down-regulated approximately 4-fold at 8 h and 10-fold at 24 and 48 h post-culture of EC cultures in 3-D collagen matrices, thus confirming that lowered levels of TG2 expression are required during the initial stages of angiogenesis. This suggests that TG2 is probably not essential to capillary formation. Given that the migration of ECs and the formation of new vessels is affected by the composition of the endothelial basement membrane, TG2 activity might be inhibitory to the endothelial mobilisation process, possibly by stabilising the endothelial basement membrane and/or causing increased cell adhesion. (Martinez et al., 1994). TG2, via its ability to cause changes in the ECM, plays a regulatory role in capillary tube development. Using in vitro and ex vivo angiogenesis assays, it was demonstrated that application of exogenous TG2 blocks angiogenesis in a dose-dependent manner without causing cell death by a mechanism that involves increased accumulation of ECM proteins (Jones et al., 2006). Matrix accumulation was accompanied by a decreased rate of ECM turnover, with increased resistance to MMP-1. Extension of these studies in vivo using transgenic melanoma and syngenic colon carcinoma tumour models demonstrated that the increased presence of TG2 in the tumor environment leads to delayed tumor growth and increased animal survival rates by a mechanism that is dependent on changes in the ECM. Additionally, by modulating matrix storage of latent TGF-β1, TG2 participates in the activation of this important wound healing cytokine (Verderio et al., 1999), possibly regulating the ECM homeostasis not only by virtue of its stabilizing function (through ECM component cross-linking) but also by regulating de novo ECM protein synthesis.

TG2 and metastasis

As defined earlier in this review an important phenotype of the highly malignant tumour cell is its ability to survive in hostile host environments as it passes through the lymphatic system or the blood stream in its attempts to colonise other host tissues. During this stage of the malignant cycle a number of selective pressures are exerted to ensure survival and colonisation. Following tumour mass maturation, the survival of malignant cells that have escaped into the vasculature further depends on their ability to dock and adhere at a distant site. Such selection pressures may explain why expression of TG2 is often higher in the secondary tumour than that found in the primary tumour (Fig. 3). TG2 expression was shown to correlate positively with the propensity of human breast carcinomas (Mehta, 1994), hemangioblastomas of the central nervous system (Mizoguchi et al., 1998) and melanomas (Fok et al., 2006) to metastasise. More recently, proteomic analysis indicated that TG2 expression was significantly up-regulated in highly metastatic PLA801D cell lines when compared to lower metastatic tumour cell lines (Jiang et al., 2003). One possible mechanism of action for the enzyme in the metastatic cascade has been proposed by Kong and Korthuis (1997), in a study based on free-floating melanoma cells in isolated arterioles, which demonstrated that TG2 stabilised tumour cell contact points with the subendothelial matrix (Kong and Korthuis, 1997).

TG2 has also been reported to act as an anti-apoptotic protein as well as a pro-apoptotic protein (Fesus and Szondy, 2005; Antonyak et al., 2006). At the cell surface TG2 plays an anti-apoptotic role whereby its association with integrins induces activation of anti-apoptotic protein Bcl-2, and could potentially activate FAK signal transduction pathways such as PI3K/Akt, and Ras/Erk (Guan, 1997). A comparable survival signalling pathway can also be induced when TG2 is deposited into the matrix and associated with FN through an RGD independent pathway involving HSPG receptors (Verderio et al., 2003). High expression of TG2 in drug resistant cells is believed to be due to promotion of the interaction between TG2, integrins and FN (Herman et al., 2006).

Apoptosis resistant cells can exhibit increased levels of TG2 expression and activity. Adriamycin resistant MCF-7 cells in particular exhibit a 40–60 fold increase in transglutaminase activity both in vitro and in vivo (Mehta, 1994). It has been hypothesised that chemoresistance in SKBR3 breast cancer cells is due to induction and activation of TG-2 expression by pro-apoptotic EGF (Antonyak et al., 2001). In keeping with this finding, MDA231/cl9-TG2 deficient cells were shown to be sensitive to doxorubicin apoptosis as opposed to MDA231/cl9-TG2 sufficient breast cancer subline (Mehta et al., 2004). Several anticancer agents, such as adriamycin, actinomycin D, mithrimycin, and bleomycicn, have been shown to serve as amine substrates for transglutaminases (Russell and Womble, 1982). A relation between efficacy of anticancer drugs, and transglutaminase activity cannot be ruled out, as the enzyme might protect against apoptosis by mopping up drugs via their covalent incorporation. Active site-directed inhibition of TG2 led to higher sensitivity in chemotherapy (apoptosis) for glioblastoma cells and tumours, with parallel decrease in prosurvival proteins (phosphorylated Akt, anti-apoptotic protein surviving) and increase in pro-apoptotic proteins such as BH3-only protein and Bim (Yuan et al., 2005).

These observations suggest that TG2 may be linked to a number of cell survival pathways evoked by the enzyme. For example NF-kappa *B* in normal cells is thought to be activated as part of the cell response to stress and trauma and is thought to orchestrate a cell survival pathway. A hostile host environment evoked by the host as tumour cells pass through the lymph system and blood stream may select for such a stress response in those tumour cells that survive. Several findings suggest that NF-kappa *B* and TG2 are very closely interconnected. A number of reports indicate that TG2 can be induced directly by NF-kappa *B* activation, which might be expected given that the TG2 promoter possesses a NF-kappa *B* binding motif. TG2 can also induce NF-kappa *B* activation via two different pathways, an IKK-independent pathway and an IKK-dependent pathway (Mirza et al., 1997; Lee et al., 2004) thus enhancing the survival signal.

Potential TG2-based antitumour strategies

This review could not be exhaustive and was instead aimed at highlighting the involvement of TG2 in specific events in tumorigenesis and metastasis that hold potential for intervention. Several essential alterations in cellular physiology collectively dictate malignant growth; insensitivity to anti-growth signals, limitless replicative potential, evasion of apoptosis, sustained angiogenesis, and tissue invasion and metastasis (Hanahan and Weinberg, 2000). Understandably, most anti-tumour therapy approaches are targeted against one or more of these tumour-specific hallmarks. Taking into account that TG2 has been implicated in a wide range of cellular functions most of which are crucial in the tumour growth process, it is possible that the enzyme might provide a tool for designing a successful anti-tumour strategy.

As outlined above, TG2 might influence tumour progression through a variety of mechanisms such as regulation of cell growth (Mian et al., 1995), cell death and survival (Mehta, 1994; Melino et al., 1994; Piacentini et al., 1996), as a cell surface receptor (Akimov et al., 2000; Verderio et al., 2003), via its involvement in ECM organisation (Barsigian et al., 1991), or through its involvement in tumour angiogenesis (Jones et al., 2006). Therefore, there is scope for implementing TG2-based tumour intervention strategies that aim to induce malignant cell death, disrupt malignant cell invasion, or inhibit angiogenesis at the site of the primary tumour.

TG2 expression is generally limited in the primary tumour body, indicating its inhibitory role in the initial tumour mass expansion. Mapping out the expression and activity profile of TG2 during the different stages of malignancy could offer useful insights on rational utilisation or modulation of its activity to block primary tumour spread. Data produced by our laboratory and others suggest that enzyme activity and expression steadily decrease as the primary tumour expands in size. However following metastasis, for reasons defined earlier, TG2 expression is generally increased.

Coming back to the thesis of this review, the question asked is whether TG2 acts as a ''friend'' or ''foe'' in the concerted events of tumour progression. Our data suggest that if TG2 expression can be maintained in the primary tumour, it is likely to act as a ''friend'' to the host provided its expression and secretion into the ECM can be maintained. Indeed, evidence from human breast carcinomas suggests that increased presence of TG2 in the stroma surrounding the primary tumour correlates to a less aggressive tumour (Mangala et al., 2005). Unfortunately the other face of TG2, ''foe'', becomes apparent in the metastatic cell where its multifunctional roles may aid cell survival and extravasation, facilitating further colonisation of the host tissue. At this point in the malignant cycle TG2 itself may be viewed as a potential therapeutic target. The roles of TG2 either as an accomplice or an opposing force in the tumour growth and metastatic cascades are summarised in Fig. 4.

A further promising cellular target for anti-invasion treatment of the primary tumour may therefore also be

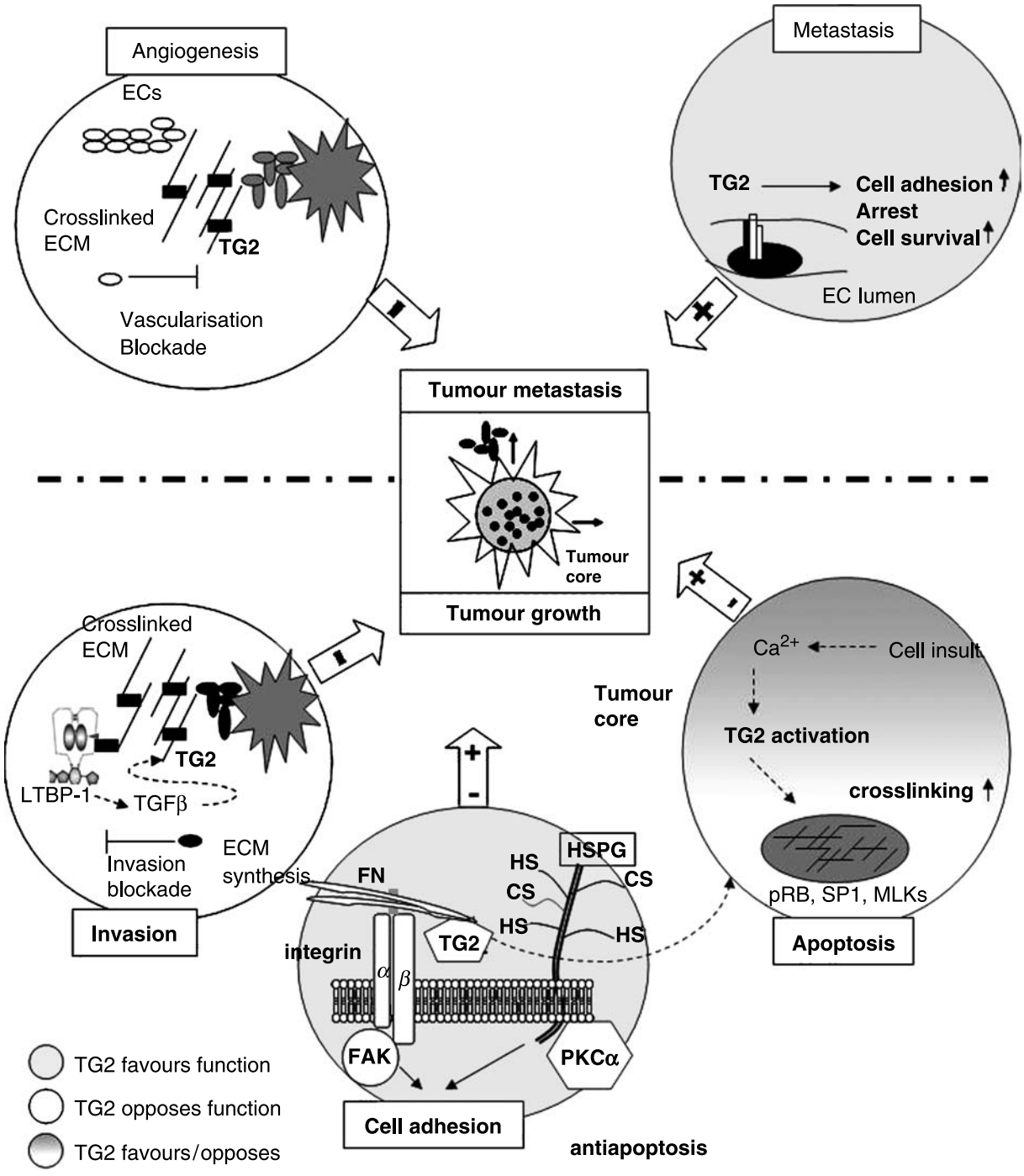

Fig. 4. The majority of cellular and biochemical functions of TG2 in the distinct processes leading to tumour progression and metastasis do not confer the enzyme a clear promoting or opposing role. TG2 can either participate in pro- or anti-apoptotic mechanisms preventing or assisting malignant cell evasion of cell death respectively. At the early stages of tumour growth when spared from MMP proteolysis, cell surface-associated TG2 promotes malignant cell adhesion through its interaction with integrins. Adhesiveness is regulated by the ECM so that freedom of tumour cell motility essential for tumour mass expansion is not compromised whilst tumour cell anoikis is prevented. Once externalised into the ECM, TG2 crosslinks ECM protein components inducing qualitative changes in ECM homeostasis, which in turn result in blockade of malignant cell invasion. In parallel TG2 mediated sequestration of LTBP-1 into the ECM makes this TGFβ precursor available for processing and release, signalling for de novo ECM synthesis. The same stable ECM also presents a formidable barrier for ECs to traverse, leading to impairment of angiogenesis. Increased TG2 in the metastatic tumour cell could provide malignant cells with increased survival characteristics to withstand the hostile host environment and when at the cell surface provide the required docking sites for arrest and colonisation of new tissue sites; ± in arrows indicate positive/negative implication of the specific TG2 function on the overall tumour growth and metastatic cascades

the stromal fibroblast and ECs that control the tumour-host microenvironment and ECM homeostasis (Liota and Kohn, 2001). Induction of fibrosis via matrix protein accumulation and crosslinking can effectively sever communication routes between host and tumour by confining malignant cells within the tumour pseodocapsule and the ECs within the stroma. In this way both tumour invasion and angiogenesis could feasibly be blocked (Jones et al., 2006; Haroon et al., 1999b).

However, the caveat is that such anti-cancer approaches if pursued in a systemic form are often subject to lack of targeted specificity to the site of malignancy. TG2 is expressed in a large number of stromal tissues where it is involved in a vast array of physiological processes as such the enzyme is best targeted to the tumour host microenvironment either topically or via intratumour injection, possibly prior to or following tumour resection. Many tumours are highly vascularised and difficult to resect by conventional surgery and as such they present good therapeutic targets for TG2-based intervention.

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