British Journal of Medicine & Medical Research
6(3): 297-311, 2015, Article no.BJMMR.2015.205
ISSN: 2231-0614

SCIENCEDOMAIN *international*
www.sciencedomain.org

The Role of Transglutaminase 2 (TG2) in Definition of Cancer Hallmarks

Odii Benedict Onyekachi[1*], U. Lawani Ebidor[2] and Omabe Maxwell[3]

[1]Department of Biomedical and Forensic Sciences, Faculty of Science and Technology, Anglia Ruskin University, East Road, Cambridge, CB1 1PT, United Kingdom.
[2]Department of Medical Laboratory Science, College of Health Science, Niger Delta University, Amassoma, Bayelsa State, Nigeria.
[3]Department of Oncology, University of Saskatchewan, Canada.

Authors' contributions

This work was carried out in collaboration between all authors. Author OBO designed the study, and wrote the first draft of the manuscript. Author ULE managed the literature searches, and author OM managed the proofreading of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2015/13919
Editor(s):
(1) Kate S Collison, Department of Cell Biology, King Faisal Specialist Hospital & Research Centre, Saudi Arabia.
Reviewers:
(1) Anonymous, Second University of Naples, Naples, Italy.
(2) Anonymous, Chaudhary Charan Singh University, India.
(3) Anonymous, University of the Witwatersrand, South Africa.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=722&id=12&aid=7274

Mini-review Article

Received 9th September 2014
Accepted 17th November 2014
Published 15th December 2014

ABSTRACT

Over the past three decades, TGM2, a stress-responsive gene encoding transglutaminase 2 (TG2) has been identified as one of the several genes that may be involved in carcinogenesis and cancer physiology. TG2 is a pleiotropic calcium-dependent enzyme belonging to the transglutaminase family of enzymes, which post-translationally modify glutaminyl and lysyl side chains on the surface of both *in vivo* and *in vitro* substrate proteins. Unlike other members of the transglutaminase family, TG2 has additional Ca^{2+}-independent enzymatic and non-enzymatic activities, which have been directly or indirectly implicated in diverse cellular physiological events, including cell growth and differentiation, cell adhesion and morphology, extracellular matrix stabilization, wound healing, cellular development, receptor-mediated endocytosis, apoptosis, and disease pathology. TG2 has specialized biochemical, structural and functional elements, wide tissue distribution and sub-cellular localisation, as well as broad substrate specificity. These specialised features of TG2 account for its multiple patho-physiological functionalities. Considering the multiplicity of TG2

**Corresponding author: Email: benedict.odii@anglia.ac.uk;*

functions and its importance in disease pathology, including cancer; we have reviewed herein, the importance of TG2 in the definition of the hallmark capabilities of cancer cells. This was done with the view to deepen our understanding of the role of TG2 in carcinogenesis and recapitulating its potential as a therapeutic target for cancer treatment.

Keywords: Transglutaminase 2; cancer; drug resistance; metastasis; apoptosis.

1. INTRODUCTION

The body of animals is analogous to a society or an ecosystem; the constituent members are cells, which reproduce by cell division and form collaborative assemblies called tissues. However, unlike a conventional human society, where survival of the fittest is the order of the day, self-sacrifice is the rule in normal cells. Thus, cells of a multicellular organism are subject to tightly regulated form of collaboration, devoid of competition and selfishness. Consequently, each cell behaves in a socially responsible manner, and must rest, grow, divide, differentiate, or die, as needed for the good of the cellular community and the organism. The behaviours of the cells are regulated by a social control network that ensures that the cells send, receive, and interpret an elaborate set of extracellular signals- this is done via the cell cycle control system [1,2]. Any attempt to disobey the societal rules by a given cell or group of cells could be disastrous for the multicellular society. Most dangerously, a successful defiance of the cell cycle control system through molecular disturbances, such as mutations may result in a given cell becoming selectively advantaged, hence, growing and dividing more vigorously and surviving more readily than neighbouring cells. This cell therefore, becomes the progenitor of a growing mutant clone, promoting selfishness among members of the cellular society as opposed to the original selflessness. Over time, this new wave of successive rounds of mutation, competition, and natural selection operating within the cellular population could degenerate to serious cellular conditions, characterised by over-proliferation - cancer [2].

Cancers are heterogeneous multicellular entities constituted by cells of multiple lineages, interacting with one another, the extracellular matrix (ECM), and soluble molecules within their vicinities in a dynamic manner that favours cell proliferation, movement, differentiation, and ECM metabolism; whilst restricting cell death, stationary polarised growth and ECM stability [1]. They are cellular diseases, especially emanating from the disruption of cellular functions either intrinsically or extrinsically. For instance, genomic alterations affecting intrinsic cellular functions, such as cell cycle check-point control, apoptosis, differentiation, metabolism, and cell adhesion; or/and those affecting the extrinsic programs, such as tissue oxygenation, matrix metabolism, immune response, and vascular status [3].

Tumorigenesis in humans is a multistep process, with each step reflecting the genetic alterations that drive the progressive transformation of normal human cells into highly malignant sub-clones. Studies of human cancers and animal models have shown that the process of tumour development is analogous to Darwinian evolutionary processes, in which a succession of genetic changes, each conferring a given type of growth advantage, results to the progressive conversion of normal human cells into cancer cells [4,5]. Hanahan & Weinberg [5] proposed that the vast catalogues of cancer cells' genotype are testaments of this succession of genetic alterations in cell physiology that lead to development of malignant phenotype. They classified such genetic alterations into six essential features, termed the hallmarks capabilities of cancer, including sustaining proliferative signalling, insensitivity to antigrowth signals, evasion of apoptosis, unlimited replicative potential, sustained angiogenesis, and tissue invasion and metastasis (see Fig. 1).

Transglutaminase 2, as a multifunctional protein with broad range of substrate specificity has been implicated in many genetic alterations in cellular physiology, hence, its undeniable involvement in determining cancer hallmark capabilities in different types of cancer. The abundant distribution of TG2 in various cells of different origins and its broad substrate specificity support its involvement in definition of many important cancer cells' physiologies that encourage selfishness. TG2-related activities have been implicated in the enhancement of cell to cell interaction, ECM stabilisation, and interaction with and modification of intracellular and extracellular proteins. These functions of TG2 favour cellular proliferation, migration,

evasion of apoptosis, and insensitivity to death signals. The involvement of TG2 in the determination of these features that define the hallmark capabilities of cancer cells is discussed in the sections below.

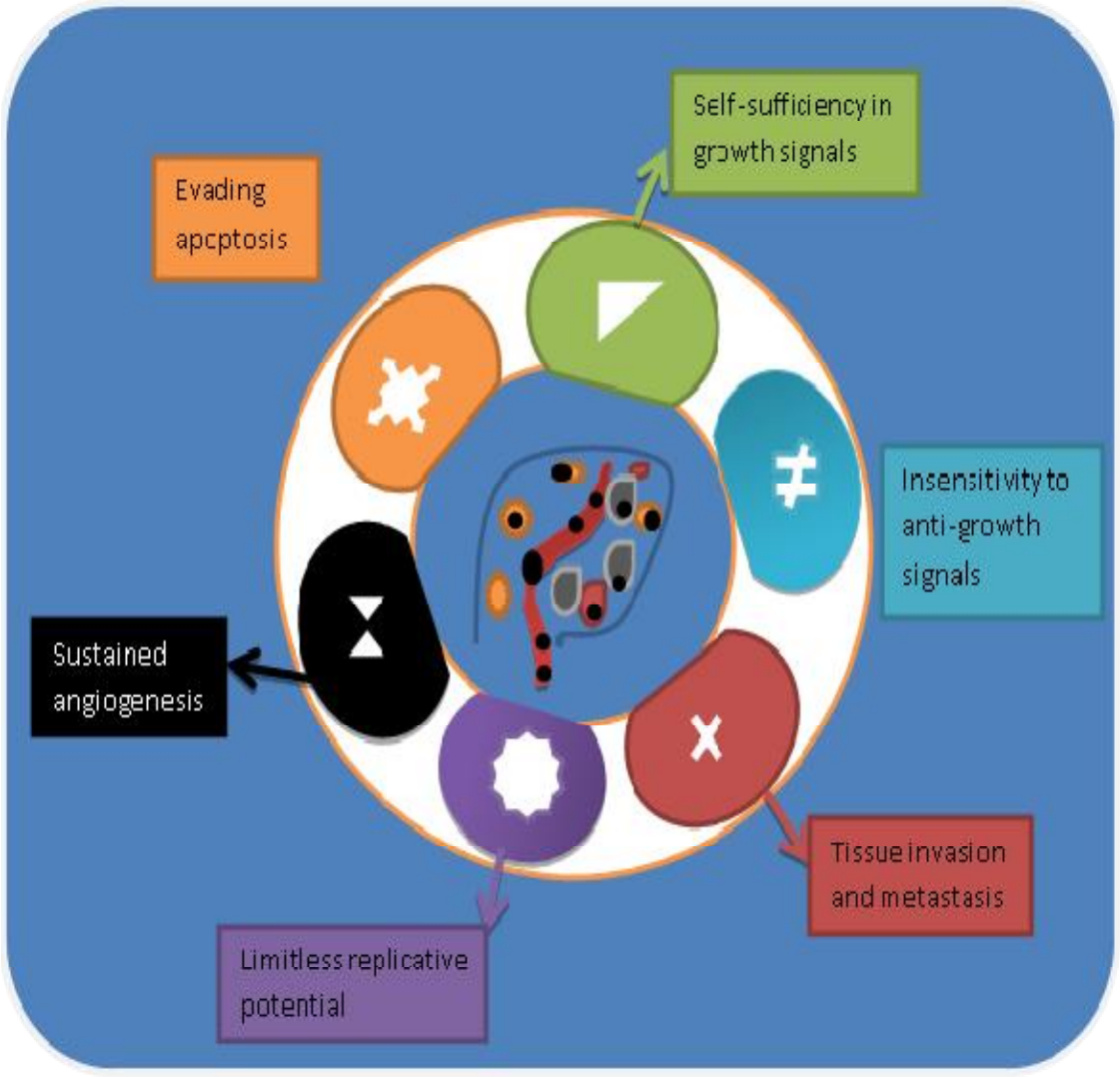

Fig. 1. The Hallmarks of cancer as proposed by Hanahan & Weinberg [5], representing the acquired capabilities of cancer cells. Tumour cells defy the cell cycle control system and become insensitive to anti-growth signals, self-sufficient in growth signals, insensitive to death signals (evade apoptosis) and uncontrollably proliferative. Consequently, mutant clones accumulate in excess of the carrying-capacity of the basement membrane of the host tissue, resulting in invasion of neighbouring tissues. The need for oxygen and nutrient through blood supply triggers development of new, defective blood vessels (angiogenesis) that encourage leakage of mutant cells to distant sites (metastasis).

2. CELLULAR AND SUBCELLULAR LOCALISATION OF TG2 AND ITS STRUCTURAL AND FUNCTIONAL ELEMENTS INVOLVED IN CANCER DEVELOPMENT

2.1 Structural and Functional Elements

Transglutaminase 2 is structurally composed of four distinct globular domains (Fig. 2): an NH2-terminal β-sandwich which contains fibronectin and integrin binding sites, a catalytic core which contains the catalytic triads (Cys277, His335 and Asp358) for acyl-transfer reaction and a conserved tryptophan essential for this catalytic reaction [6], and two COOH-terminal β-barrel domain with the second barrel domains containing a phospholipase C binding sequence [7,8].

Unlike other transglutaminase enzymes, TG2 possesses a distinctive guanidine nucleotide-binding site, located in the cleft between the catalytic core and the first β-barrel (Fig. 2) [8], this sequence is coded by exon 10 of the TG2 gene, which is characterised by lower sequence homology with the same exons in other transglutaminases. Some GDP/GTP-interacting residues and those necessary for GTP hydrolysis are situated in other domains [9]. In the GDP-bound form of TG2, access to the transamidation active site is blocked by two loops, and the active site cysteine is attached to a tyrosine residue by hydrogen bonding. In the latent conformation of TG2, there is a significant inter-domain interaction between the catalytic domain 2 and domains 3 and 4, which reduces the accessibility of the active centre [10].

The structural conformation of TG2 in its Ca^{2+}-bound form is yet to be resolved. A putative Ca^{2+}-binding site, homologous to the one demonstrated in FXIIIA [11], is distorted in the TG2 structure by the bound nucleotide [8]. The binding of Ca^{2+} to the catalytic domain of TG2 alters the conformation of proteins as domains 3 and 4 are moved further apart from the catalytic domain, thus making the active site of TG2 accessible [8,12]; the hydrogen-bonded tyrosine is also displaced in the process [13]. The ability of GTP to inhibit the transamidation activity of TG2 is determined by the potential of GTP to bind and subsequently hydrolyse Ser171 and Lys173 residues of the second domain [9].

2.2 TG2 Distribution in Cellular and Sub-cellular Locations

The cellular distribution of TG2 is ubiquitous, with its expression levels highest in endothelial cells and monocyte-derived macrophages; although, it is significantly expressed in vascular smooth muscle cells, connective tissue fibroblasts, osteoblasts, neurons, hepatocytes, astrocytes, and epidermal keratinocytes [10,14].

Transglutaminase 2 is constitutively expressed in different types of cells, while in some other cells its expression is induced by external stimuli or as part of their differentiation/maturation [15]. At the cellular level, TG2 is localized both inside the cell and on the cell surface as shown by the schematic representation in Fig. 3. The intracellular location of TG2 is predominantly in the cytosol, however it has also been reported to be present in the nucleus and associated with the mitochondria [16]. As a result of low concentration of Ca^{2+} within the cytoplasm, the transamidating activity of TG2 is thought to remain dormant inside the cell, while the protein functions as a GTPase [10,17]. However, cytosolic TG2 can be activated by most cellular stressors which trigger extracellular calcium ion influx or release of calcium ion from the intracellular stores [15]. The nuclear localisation of TG2 has been reported to be approximately 5% or less [18]. Cytosolic TG2 migrates to the nucleus in response to specific stimuli [19], and importin-3 is responsible for its translocation into the nucleus [20]; where it can either function as a G-protein [21] or as a transamidase activated by nuclear Ca^{2+} signals to cross-link histones [22].

A significant proportion of TG2 is found in association with membranes of different cell types [23]. The localisation of TG2 on the surfaces of various cells types as well as in the extracellular matrix has been established [24]. Irrespective of the lack of a leader sequence or transmembrane domain, which would have helped in the translocation of TG2 to the surface by the conventional endoplasmic recticulum/golgi route, the enzyme is secreted from cells in a controlled manner, through unknown mechanisms [25,26,27].

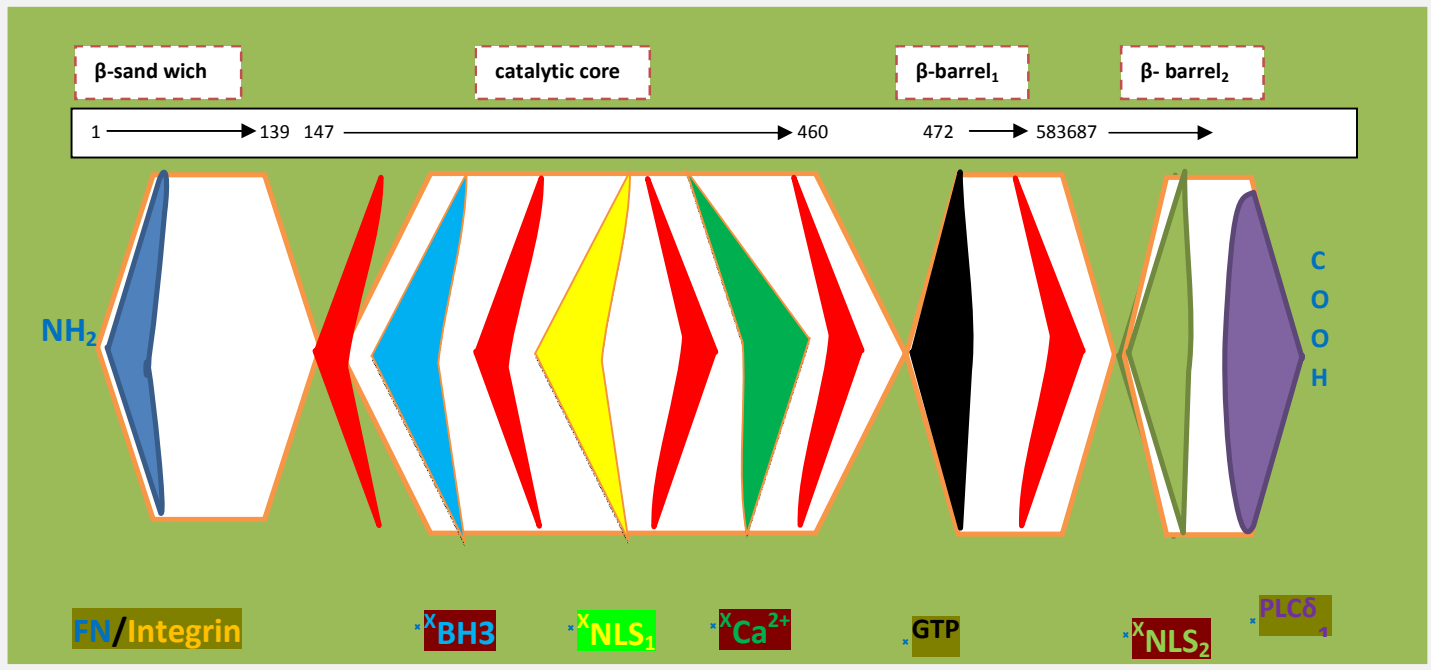

Fig. 2. Schematic representation of the functional elements of TG2 indicating the four structural domains (arrows) and amino acid positions (top), with the different functional regions indicated: fibronectin/integrin binding site (FN/integrin), binding site for pro-apoptotic BH3-only protein, nuclear localisation sequences 1 and 2 (NLS1 and NLS2), calcium binding site (Ca2+), GTP binding site, and phospholipase C (PLC) receptor site [10]

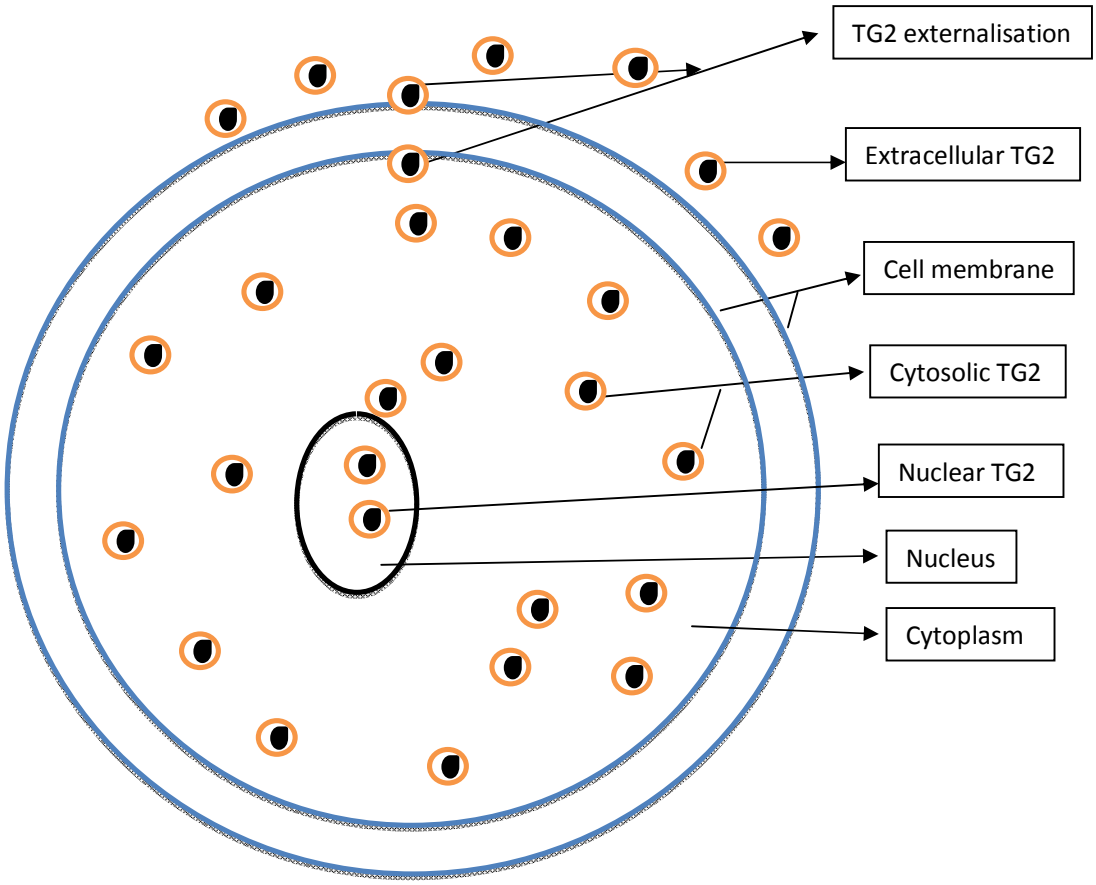

Fig. 3. Cellular distribution of TG2 (black dot in yellow circle): TG2 is localised in the nucleus (nuclear TG2), cytoplasm (cytosolic TG2), and cell surface (extracellular TG2). It is translocated into the nucleus through the help of importin, while TG2 externalisation to the cell surface occurs through unknown mechanisms.

On externalization, cell surface TG2 has been shown to facilitate cellular interactions with the surrounding extracellular matrix (ECM); which are critical physiological processes underlying many key aspects of cell behaviour, including cell adhesion, growth, migration, differentiation, programmed cell death, and ECM assembly [15]. In turn, these cellular processes are vital to embryogenesis and tissue morphogenesis, wound healing and tissue repair, as well as tumour growth and metastasis. Gentile et al. [28] first suggested the involvement of transglutaminase 2 in the mediation of cell-matrix adhesion. They investigated the effect of TG2 over-expression on the spreading of fibroblasts and their increased resistance to trypsinization. Subsequent convincing proofs at both cellular and molecular levels support involvement of TG2 in the mediation of cellular interactions with ECM and it has been demonstrated that TG2 serves as an adhesion receptor for fibronectin (FN) on the cell surface [29,30,31,32].

3. TG2 INTERACTION WITH FIBRONECTIN (FN) AND INTEGRIN: IMPLICATION IN CELL ADHESION AND SURVIVAL

Pathologically, FN is profoundly involved in wound healing, inflammation, blood clotting and thrombosis, as well as tumour growth and angiogenesis [15]. FN in its polymeric form, is represented in the extracellular matrix by fibrillar matrices [33], which not only promotes cell adhesion, but as well serves as a scaffold for assembly of other ECM molecules; and provide important orientations for surrounding cells, initiating cascades of signals upon interaction with cell surface receptors [34,35].

TG2 has very high affinity for FN, to which it has been shown bind at 2:1 stoichiometry [36], independent of either Ca^{2+} or the transamidating and GTPase activities of TG2 [37]. The interaction of extracellular TG2 with FN has been shown to be involved in cell-matrix adhesion [30] and many other adhesion-dependent phenomena, such as cell migration, matrix assembly and signalling [38,39]. The gelatin-binding domain (42kD) serves as the only binding site for TG2 on FN and binds TG2 with similar affinity as the whole FN [40]. Furthermore, the adhesive function of TG2 is favoured by the fact that the 42kD gelatin-binding domain of FN contains no interaction sites for the numerous FN-binding integrins, as well as other FN-associated adhesion receptors [41]. Therefore, TG2 and integrin can independently bind distinct domains of FN, consequently existing in collaboration rather than engaging in competition in the cell adhesion process [15]. It has been established in different cell types that the binding of TG2 to the 42kD fragment of FN results in stable cell adhesion, limited spreading and formation of specialized adhesive structures at the cell-substrate interface [31,38].

Irrespective of the co-existence of TG2 and integrin at different FN-binding domians, where they streamline the cell adhesion process; TG2 also associates with integrins to maintain cell-extracellular matrix (ECM) interactions. This has been demonstrated in different cell types, where transglutaminase 2 has been shown to interact with many integrin receptors, by binding to the extracellular domains of the β1 and β3 integrin subunits [30,31,38].

The stable non-covalent TG2-integrin complexes are formed independent of the transamidating activity of TG2, and there is no evidence of integrin serving as enzymatic substrate of TG2 or other transglutaminases [30]. The ability of TG2 to form ternary adhesive complexes with integrins and FN, where all the three proteins successfully interact with each other [15], highlights the importance of TG2 effects on cell adhesion and indicates an unconventional role of TG2 as a co-receptor in cell-matrix interactions and cell survival [30].

4. IMPLICATIONS OF TG2 IN CANCER ACQUISITION OF SELF-SUFFICIENCY IN GROWTH SIGNALS

Normal cells typically move from quiescent state into active proliferative state only when there is adequate supply of necessary mitogenic growth signals. These signals are transmitted into the cell by transmembrane receptors that interact with various classes of signalling molecules, including diffusible growth factors, ECM components, and inter-cell adhesion/interaction molecules, including TG2 [42]. However, the role of TG2 in growth promotion and maintenance of self-sufficiency in tumour cells could be attributed to its activation of the growth factor, transforming growth factor beta (TGFβ), resulting in promotion of cell growth and survival. Furthermore, TG2 can be involved in tumour growth sufficiency through its interactions with various adhesion molecules, including integrin and fibronectin, resulting in stabilization of extracellular matrix and activation of cell survival signalling [43]. The production and release of growth-promoting signals are carefully controlled in normal tissues, ensuring the homeostasis of cell number and maintenance of normal tissue structure; whilst entering into and progressing through the cell growth and division cycles [44]. One of the fundamental features of cancer cells is their acquired ability to sustain proliferation, as they mostly show reduced dependence on stimulation from their normal tissue microenvironment. They maintain self-sufficiency in growth signal by dysregulating the mitogenic signals to their own advantage; thus, becoming independent of exogenous signals [5,42].

5. TG2 IN TUMOUR INSENSITIVITY TO ANTIGROWTH SIGNALS

To maintain cellular quiescence and tissue homeostasis, myriads of anti-proliferative signals operate within a normal tissue. These antigrowth signals include both soluble growth inhibitors and immobilised inhibitors both in the ECM and on the surfaces of adjacent cells. They are received by transmembrane cell surface receptors within the intracellular signalling circuits; inhibiting proliferation via two discrete mechanisms. One mechanism involves forcing cells into quiescent (G_0) state, from which they could regain proliferative feature when the extracellular environment becomes favourable. Alternatively, cells may be compelled to infinitely relinquish their proliferative potentials by being induced into post-mitotic state [5,45].

Besides their acquired capability of inducing and sustaining proliferation-promoting signals, cancer cells have the tendency to evade anti-proliferative signals. Much of the circuitry that determines the ability of normal cells to respond

to antigrowth signals is associated with the cell cycle clock, especially the parts governing cellular transit through the G_1 phase of its growth cycle. During this period, cellular decision to enter into proliferative or quiescent or post-mitotic state is dependent on the sensed signals from the external environment [5]. At molecular level, most anti-proliferative signals are funnelled through the retinoblastoma protein (pRb), which is regulated by nuclear TG2 [46]. In a hypophosphorylated state, pRb inhibits proliferation by altering the functions of transcription factors responsible for controlling the expression of catalogue of genes necessary for transition from G_1 to S phase of the cell cycle [47,48]. Additionally, TG2 has been shown to modulate pRb, depending on its phosphorylation state, leading to cell cycle arrest [49] and possible transition to quiescence.

6. TUMOUR CELLS' EVASION OF APOPTOSIS: IMPLICATIONS OF TG2

Over the past two decades, the idea that programmed cell death by apoptosis naturally serves as a barrier to cancer development, has been established by previous studies [44, 50]. Elucidation of the signalling pathways of apoptosis has revealed how apoptosis is ignited in response to various physiologic stresses undergone by cancer cells in the course of tumorigenesis, or those due to anticancer therapy. Such apoptosis-inducing stresses include signalling imbalances emanating from elevated levels of oncogene signalling, and DNA damage associated with hyper-proliferation. However, other research has shown apoptosis is attenuated in those tumours that successfully progress to advanced states of malignance and resistance to therapy [50,51].

Cancer cells can acquire the ability to resist apoptosis through various strategies. The most prominent strategy is through the loss of p53 tumour suppressor function, with the resultant removal of a key component of the DNA damage sensor capable of inducing the apoptotic cascade [52]. Alternatively, tumours may adopt the strategy of increasing expression of anti-apoptotic regulators (Bcl-2, Bcl-x_L) or of survival signals, by down-regulating pro-apoptotic factors (Bax, Bim, Puma), or short-circuiting the extrinsic ligand-induced death route. The multiplicity of apoptosis-evading mechanisms serves as a reflector of the diversity of apoptosis-inducing signals encountered by cancer cell populations during their transition to the malignant state [44].

Transglutaminase 2 has been shown to be involved in these multiple apoptosis-evading mechanisms. For instance, Boehm et al. [49] reported that nuclear TG2 exerts anti-apoptotic effect by up-regulating retinoblastoma protein pRb, leading to the polymerization of the alpha-inhibitory sub-unit of the transcription factor NF-kappaβ and concomitant cell protection from apoptosis with the help of other key anti-apoptotic proteins (Fig. 4). Also, TG2 can translocate to the plasma membrane where it serves as a co-receptor for integrin, promoting its interaction with fibronectin, resulting in the activation of cell survival and anti-apoptotic signaling pathways as reviewed in [53].

7. ACQUISITION OF UNLIMITED REPLICATIVE POTENTIAL BY CANCER CELLS: IMPLICATIONS OF TG2

For cancer cells to generate macroscopic tumours, they require unlimited replicative potential [44]; which is dependent on three acquired capabilities – growth signal autonomy, insensitivity to antigrowth signals, and apoptotic resistance, all of which lead to an uncoupling of cell's growth program from the prevailing signals in its environment [5]. The unlimited replicative capability of cancer cells remarkably contrasts the behaviour of the cells in most normal cell lineages in the body, which are only able to pass through a limited number of successive cell growth-and-division cycles [44]. This limited replication ability exhibited by normal cells is mediated by two distinct barriers to cell proliferation: senescence, cell transition to irreversible non-proliferative but viable state, and crisis, which involves cell death [54].

When cells are propagated in culture, cellular senescence is first induced by repeated cell division cycles and subsequently, cells that able to circumvent senescence will enter crisis phase, in which most of the cells in the population die. Rarely, cells from a population in crisis survive and assume unlimited replicative potential - immortalization, a feature possessed by most established tumour cell lines due to their ability to proliferate in culture without evidence of senescence or crisis [44,54]. This is an indication that limitless replicative potential (immortalization) is a phenotype acquired by cancer cells *in vivo* during tumour progression and could be vital to their development into malignant growth state [54]. By implication, at some point during the course of multistep tumour

progression, developing premalignant cell populations usually resort to evasion of mortality barrier, and assume unlimited replication so as to achieve tumorigenesis.

Transglutaminase 2 has been widely reported to enhance cancer cells' development of stem cell phenotype, through the induction of epithelial mesenchymal transition (EMT) and consequent activation of survival signalling molecules, including FAK, Akt, and NF-ƙβ as reviewed in [55]. Additionally, Kumar et al. [56,57] reported that TG2-expressing mammary epithelial cells showed increased tendency to form mammospheres, self-renewal ability, and plasticity (unlimited replication). Consequently, Agnihotri et al. [58] suggested that sustained expression of TG2 leads to the induction of EMT and stem cell-like characteristics in breast cancer cells, contributing to development of drug-resistant and metastatic phenotypes.

8. TG2 IN ANGIOGENESIS

In normal tissues, oxygen and nutrients supplied by the vasculature are essential for cell survival and function; hence, it is obligatory for virtually all cells in a tissue to reside within 100μm of a capillary blood vessel [5]. Tumour micro-environments are mostly characterised by poor vascularisation and consequent deficiency in oxygen and nutrient supplies. However, like

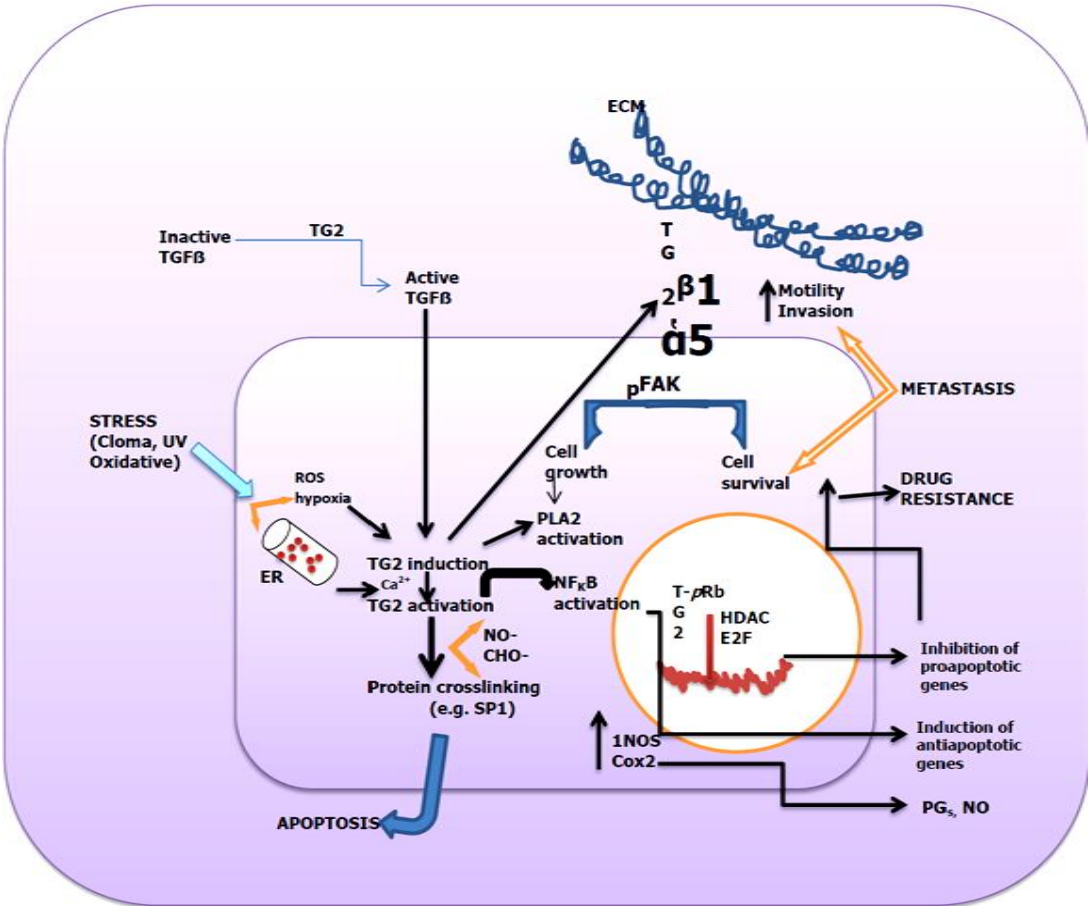

Fig. 4. Mechanisms of TG2-mediated pro-apoptosis and anti-apoptosis. In the presence of cellular stressors such as chemotherapy or UV radiation, release of intracellular Ca^{2+} from endoplasmic reticulum (ER) results in the activation of TG2 and intracellular protein crosslinking. Consequently, apoptosis is initiated and cellular contents are prevented from spillage, hence inflammation is prevented. Conversely, the activation of TG2 can result in concomitant activation of $NF_K\beta$ and induction of anti-apoptotic genes and inhibition of pro-apoptotic genes [43]

normal tissues, tumours require sustenance in the form of oxygen and nutrients just as they need to get rid of wastes and carbon dioxide [44]. Consequently, tumours tend to abrogate these deficiencies by generating tumour-associated neo-vasculature through the process of angiogenesis.

During embryogenesis, vasculature development involves the birth and assembly of new endothelial cells into tubes, in addition to the development of new vessels from pre-existing ones. Subsequent to this morphogenesis, the normal vasculature becomes largely quiescent [44]. As part of the physiologic processes in the adult, as in the cases of female reproductive cycling and wound healing, angiogenesis is transiently turned on. However, the process of tumour progression contrasts the transient switching which occurs in normal physiological scenario, as an angiogenic switch is almost always activated and remains on, resulting in normally quiescent vasculature to resort to sustained angiogenesis in order to keep with the needs of expanding tumour growth [59,60].

The formation of new blood vessel is dependent on changes in the behavioural features of endothelial cells, particularly their proliferation, migration, and differentiation into tubular structures, which is influenced by changes in the ECM [61]. Transglutaminase 2 is abundantly distributed in endothelial cells [62], and there have been many reports suggesting the importance of TG2 in the angiogenic process [63]. It is well known that many ECM proteins serve as TG2 substrates [64] and the crosslinking of these proteins by endothelial cells' TG2 result in the stabilisation of the basement membrane [65]. Recently it was demonstrated that the crosslinking activities of TG2, especially involving ECM proteins, have substantial implications in angiogenesis [66].

9. TRANSGLUTAMINASE 2 IN CANCER DRUG RESISTANCE, INVASION AND METASTASIS

During tumour development, aggregate of primary tumours tend to amass within the confines of the basement membrane of the host tissue until the carrying-capacity of the membrane is exceeded, with resultant breakage of the membrane. Consequently, neighbouring tissues are invaded by the tumours, which thence, migrate to distant sites where they may successfully establish as new colonies – metastasis [2]. The invasive and metastatic capabilities of cancer cells enable them to escape the primary tumour site and colonise new body areas devoid of nutrient deficiency and space limitation. Similar to the primary tumour formation, successful invasion and metastasis are dependent on other acquired hallmark capabilities [5].

Exhibition of apoptotic resistance is a common characteristic of advanced cancers [67]. This feature does not only give the tumour cells the ability to metastasise but also the ability to develop a drug-resistant phenotype [68]. In essence, drug resistance and metastasis share many features in common. For example, tumour cells selected for drug resistance *in vitro* are more metastatic *in vivo.* Conversely, metastatic tumours generally show higher resistance to chemotherapy than their primary counterparts [55]. Transglutaminase 2 is involved in the modulation of apoptosis and cell fate through many crucial cellular functions (as reviewed in the previous section). When aberrantly regulated, TG2 is thought to have a role in cancer cell's ability to evade apoptosis. Evidently, there seems to be direct connection of TG2 with cancer drug resistance [69,70] and mechanism of metastatic progression [71].

Many studies have demonstrated elevated TG2 expression as a hallmark of many types of cancer cells, including pancreatic carcinoma [72], ovarian carcinoma [73,74], malignant melanoma [75], lung carcinoma [76], glioblastoma [77], and breast carcinoma [71]. For instance, [78] on analysing the genes from tumour samples observed that out of over 30,000 genes analysed, TG2 was among those that recoded the highest expression in pancreatic carcinoma. Similarly, Jiang et al. [79], while attempting to identify metastasis-associated proteins through proteomic analysis, observed that TG2 was one of the eleven proteins that were constitutively elevated in metastatic human lung carcinoma. In another development, Antonyak et al. [80] showed that cancer cells treated with epidermal growth factor (EGF) expressed high level of TG2 and were consequently, protected cells from doxorubicin-induced apoptosis. These observations are strong reflectors of the implications of aberrant TG2 expression in the conferment of apoptotic resistance and consequent drug resistance and metastatic potentials of cancer cells.

Furthermore, Park et al. [81] reported that TG2-specific cross-linking activity resulted in the polymerization and inhibition of nucleophosmin, and concomitant increase in drug resistance potential of cancer cells. Recent evidence shows that aberrant expression of TG2 in mammary epithelial cells bestows stem cell characteristics on the cells [56]. Similarly, Kumar and colleagues reported that high basal expression of TG2 in breast cancer cells promotes the development of stem cell features, but did not encourage their terminal differentiation [56]. Additionally, Caffarel et al. [82] observed that the activation of TG2: integrin-α5ß1 interactions through the stimulation of oncostatin M receptor in cervical squamous cell carcinoma, induced pro-malignant changes.

Clinically, TG2 has been reported to serve as a predictive indicator of anticancer therapeutic efficacy. For instance, Jae-HeonJeong et al. [83] suggested that TG2 expression is a promising indicator of the effectiveness of epidermal growth factor receptor tyrosine kinase inhibitor (EGFR-TKI) therapy in patients suffering from non-small cell lung cancer. Similarly, Assi et al. [84] reported that the accumulation of TG2 in tumourstroma can serve as an independent risk factor for the identification of invasive ductal carcinomas (IDCs) of breast, and can establish breast cancer patients at high risk of recurrence. They also observed that overexpression of TG2 can serve as an indicator of poor prognosis for IDC of the breast. Agnihotri et al. [22] proposed that inflammation-induced progression of breast cancer and acquisition of survival and invasive capabilities by breast cancer cells are mediated by TG2. In acute myeloid leukemia, [85] demonstrated that increased expression of TG2 characterized a more advanced state of the disease in relapse patients. They further established that increased TG2 expression correlates with the expression of proteins involved in apoptosis, motility and extracellular matrix association; processes that have been linked with leukemia development and progression.

10. DOWN-REGULATION OF TG2 EXPRESSION AND INHIBITION OF ACTIVITY: IMPLICATIONS IN CANCER

TG2 down-regulation or inhibition by small interfering RNA (siRNA), antisense RNA, ribozyme, or small molecule inhibitors have been shown to increase the susceptibility of various cancer cell types to chemotherapy-induced cell death, and to inhibit invasion, both *in vitro* and *in vivo* [74,86,87]. Satpathy et al. [73] observed that increased TG2 expression promoted the adhesion of ovarian cancer cells to fibronectin and facilitated directional cell migration, while TG2 down-regulation in similar cells decreased tumour dissemination on the peritoneal surface and in mesentery in an intra-peritoneal ovarian xenograft mouse model. Put together, these observations strongly support that over-expression of TG2 confers resistance to chemotherapeutic drugs and promotes the invasive potential of malignant cells.

Recently, Wang et al. [66] reported that angiogenesis is attenuated in cell culture, the aorta ring assay and *in vivo* models following the inhibition of the crosslinking activity of extracellular TG2 or down-regulation of its expression. They further posited that inhibition of the activity of extracellular TG2 in human umbilical vein endothelial cell (HUVEC) co-culture model can halt angiogenic progression, even after the commencement of tubule formation and in the presence of excess vascular endothelial growth factor (VEGF). Additionally, Wang and colleagues suggested that down-regulation of TG2 expression by short hairpin (shRNA) inhibited HUVEC migration and tubule formation [66], hence, TG2-related activity has angiogenic role.

Down-regulation of TG2 protein expression by siRNA interference enhances the susceptibility of drug-resistant hepatocarcinoma (HEPG2) cells to cisplatin and 5-fluourouracil treatment, and leads to reduced invasion and migration potential of parental and drug-resistant HEPG2 cells on matrigel-coated surface. Comparatively, the inhibition of TG2 activity using cystamine profoundly increased chemosensitivity of parental and drug-resistant HEPG2 cells and attenuated their potential to invade and migrate through matrigel-coated surface [88].

11. CONCLUSION

Gene regulation determines enzyme availability and level of activity. Consequently, increase in TG2 protein expression leads to increase in its enzymatic activity, with resultant increase in cancer drug resistance and metastasis; depending on cell type and stimulation. From the foregoing review, it is glaring that TG2-mediated activities are important in the definition of many hallmark capabilities of cancer cells. The role of TG2 in cancer biology could be ascribed to its ubiquitous tissue and cellular distribution, broad

substrate specificity and multiple functionalities. This Mini-review, therefore, highlights the importance of TG2 in carcinogenesis.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Tlstyl TD, Coussens LM. Tumour stroma and regulation of cancer development. Annu. Rev. Pathol. Mech. Dis. 2006;1:119–50.
2. Albert B, et al. Molecular biology of the cell. 5th Ed. Garland science, Taylor & Francis Group. New York, USA. 2008;1205-1266.
3. McCormick, F. Cancer: Survival pathways meet their end. Nature. 2004;428:267–69.
4. Nowell PC. The clonal evolution of tumour cell populations. Science.1976;194:23–28.
5. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000;100:57–70.
6. Murthy SN, et al. Conserved tryptophan in the core domain of transglutaminase essential for catalytic activity. Proc. Natl. Acad. Sci. 2002;99:2738–2742.
7. Hwang K-C, et al. Interaction site of GTP bindingGh (transglutaminase II) with phospholipase C. J. Biol. Chem. 1995;270:27058–27062.
8. Liu S, Cerione RA, Clardy J. Structural basis for the guanine nucleotide-binding activity of tissue transglutaminase and its regulation of transamidation activity. Proc. Natl. Acad. Sci. 2002;99:2743–2747.
9. Iismaa SE, et al. GTP binding and signalling by Gh / transglutaminase II involves distinct residues in a unique GTP-binding pocket. J. Biol. Chem. 2000;275:18259–18265.
10. Fesus L, Piacentini M. Transglutaminase 2: An enigmatic enzyme with diverse functions.Trends in Biochemical Sciences. 2002;27(10):534-539.
11. Fox BA, et al. Identification of the calcium binding site and a novel ytterbium site in blood coagulation factor XIII by X-ray crystallography. J. Biol. Chem. 1999;274:4917–4923.
12. Mariani P, et al. Ligand-induced conformational changes in tissue transglutaminase: Monte Carlo analysis of small-angle scattering data. Biophys. J. 2000;78:3240–3251.
13. Noguchi, K. et al. Crystal structure of red sea bream transglutaminase. J. Biol. Chem.2001;276:12055–12059.
14. Lorand L, Graham RM. Transglutaminases: Crosslinking enzymes with pleiotropic functions. Nat. Rev. Mol. Cell Biol. 2003;4(2):140-56.
15. Zemskov EA, et al. The role of tissue transglutaminase in cell-matrix interactions. Frontiers in Bioscience. 2006;11:1057-1076.
16. Telci D, Griffin M. Tissue transglutaminase (TG2) - a wound response enzyme. Frontiers in Bioscience. 2006;11: 867-882.
17. Nakaoka H, et al. Gh: A GTP-binding protein with transglutaminase activity and receptor signalling function. Science. 1994;264:1593-1596.
18. Lesort M, Attanavanich K, Zhang J, Johnson GV. Distinct nuclear localization and activity of tissue transglutaminase. J. Biol. Chem. 1998;273:11991-11994.
19. Milakovic T, Tucholski J, McCoy E, Johnson GV. Intracellular localization and activity state of tissue transglutaminase differentially impacts cell death. J. Biol. Chem. 2004;279:8715-8722.
20. Peng X, et al. Interaction of tissue transglutaminase with nuclear transport protein importin-α3. FEBS Lett.1999;5:35-39.
21. Singh US, et al. Identification and biochemical characterization of an 80 kilodalton GTP-binding/transglutaminase from rabbit liver nuclei. Biochemistry. 1995;34:15863–15874.
22. Ballestar E, et al. Conformational changes in the nucleosome followed by the selective accessibility of histone glutamines in the transglutaminase reaction: Effects of salt concentrations. Biochemistry. 2001;40:1922–1929.
23. Aeschlimann D, Thomázy V. Protein crosslinking in assembly and remodelling of extracellular matrices: The role of transglutaminases. Connect. Tissue Res. 2000;41:1-27.

24. Upchurch HF, Conway E, Maxwell MD. Localization of cellular transglutaminase on the extracellular matrix after wounding: Characteristics of the matrix-bound enzyme. J. Cell. Physiol., 1991;149:375-382.
25. Gentile V, et al. Isolation and characterization of CDNA clones to mouse complexes. J. Biol. Chem. 1991;267:7880-7885.
26. Gaudry CA, et al. Cell surface localisation of tissue transglutaminase is dependent on a fibronectin-binding site in its N-terminal b-sandwich domain. J. Biol. Chem. 1999;274:30707-30714.
27. Di Venere A, et al. Opposite effects of Ca^{2+} and GTP binding on tissue transglutaminase tertiary structure. J. Biol. Chem. 2000;275:3915-3921.
28. Gentile V, et al. Expression of tissue transglutaminase in Balb-C 3T3 fibroblasts: Effects on cell morphology and adhesion. J. Cell Biol. 1992;119:463-474.
29. Verderio E, Nicholas B, Gross S, Griffin M. Regulated expression of tissue transglutaminase in Swiss 3T3 fibroblasts: Effects on the processing of fibronectin, cell attachment, and cell death. Exp. Cell Res. 1998;239:119-138.
30. Akimov SS, Krylov D, Fleischman LF, Belkin AM. Tissue transglutaminase is an integrin-binding adhesion co-receptor for fibronectin. J. Cell Biol. 2000;148:825-838.
31. Belkin AM, et al. Matrix-dependent proteolysis of surface transglutaminase by membrane-type metalloproteinase regulates cancer cell adhesion and locomotion. J. Biol. Chem. 2001;276:18415-18422.
32. Kabir-Salmani M, et al. Tissue transglutaminase at embryo-maternal interface. J. Clin. Endocrinol. Metab. 2005;90:4694-4702.
33. Wierzbicka-Patynowski I, Schwarzbauer JE. The ins and outs of fibronectin assembly. J. Cell Sci. 2003;116:3269-3276.
34. Pereira M, et al. The incorporation of fibrinogen into extracellular matrix is dependent on active assembly of a fibronectin matrix. J.Cell Sci. 2002;115:09-617.
35. Sottile J, Hocking DC. Fibronectin polymerization regulates the composition and stability of extracellular matrix fibrils and cell-matrix adhesions. Mol. Biol. Cell. 2002;13:3546-3559.
36. LeMosy EK, et al. Visualization of purified fibronectin transglutaminase macrophage and human endothelial cell tissue transglutaminase. J. Biol. Chem. 1992;266:478-483.
37. Turner PM, Lorand L. Complexation of fibronectin with tissue transglutaminase. Biochemistry,1989;28:628-635.
38. Akimov SS, Belkin AM. Cell-surface transglutaminase promotes fibronectin assembly via interaction with the gelatin-binding domain of fibronectin: A role in TGFbeta-dependent matrix deposition. J. Cell Sci. 2001;114:2989-3000.
39. Verderio EAM, et al. A novel RGD-independent cell adhesion pathway mediated by fibronectin-bound tissue transglutaminase rescues cells from anoikis. J. Biol. Chem. 2003;278:42604-42614.
40. Radek JT, et al. Affinity of human erythrocyte transglutaminase for a 42-kDa gelatin-binding fragment of human plasma fibronectin. Proc. Natl. Acad. Sci. USA. 1993;90:3152-3156.
41. Hang J, Zemskov EA, Lorand L, Belkin AM. Identification of a novel recognition sequence for fibronectin within the NH2-terminal β-sandwich domain of tissue transglutaminase. J. Biol. Chem. 2005;280:23675-23683.
42. Witsch E, Sela M, Yarden Y. Roles for growth factors in cancer progression. Physiology. 2010;25:85–101.
43. Mehta K, Fok JY, Mangala LS. Tissue transglutaminase: From biological glue to cell survival cues. Frontiers in Bioscience. 2006;11:163-185.
44. Hanahan D, Weinberg R A. Hallmarks of cancer: The next generation. Cell. 2011;144:646-674.
45. Deshpande A, Sicinski P, Hinds PW. Cyclins and cdks in development and cancer: A perspective. Oncogene. 2005;24:2909–2915.
46. Kuo T, Tatsukawa H, Kojima S. New insights into the functions and localization of nuclear transglutaminase 2, FEBS Journal. 2011;278:4756–4767.
47. Weinberg RA. The retinoblastoma protein and cell cycle control. Cell. 1995;81:323–330.
48. Burkhart DL, Sage J. Cellular mechanisms of tumour suppression by the retinoblastoma gene. Nat. Rev. Cancer. 2008;8:671–682.

49. Boehm JE, et al. Tissue transglutaminase protects against apoptosis by modifying the tumour suppressor protein P110 Rb. J. Biol. Chem. 2002;277:20127–20130.
50. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene. 2007;26:1324–1337.
51. Lowe SW, Cepero E, Evan G. Intrinsic tumour suppression. Nature. 2004;432:307–315.
52. Harris CC. P53 tumour suppressor gene: From the basicresearch laboratory to the clinic—an abridged historical perspective. Carcinogenesis.1996;17:1187–1198.
53. Odii BO, Coussons P. Biological functionalities of transglutaminase 2 and the possibility of its compensation by other members of the transglutaminase family. The Scientific World Journal; 2014. Available:http://dx.doi.org/10.1155/2014/714561
54. Hayflick L. Mortality and immortality at the cellular level: A review. Biochemistry.1997;62:1180–1190.
55. Mehta K, Kumar A, Kim H. Transglutaminase 2: A multi-tasking protein in the complex circuitry of inflammation and cancer. Biochemical Pharmacology. 2010;80:1921–1929.
56. Kumar A, et al. Evidence that aberrant expression of tissue transglutaminase promotes stem cell characteristics in mammary epithelial cells. PLoS ONE. 2011;6(6):e20701. DOI:10.1371/journal.pone.0020701.
57. Kumar A, Xu J, Sung B, Kumar S, Yu D, Aggarwal BB, Mehta K. Evidence that GTP-binding domain but not catalytic domain of transglutaminase 2 isessential for epithelial-to-mesenchymal transition in mammary epithelial cells. Breast Cancer Res. 2012;14:R4.
58. Agnihotri N, Santosh Kumar, Kapil Mehta. Tissue transglutaminase as a central mediator in inflammatory-induced progression of breast cancer. Breast Cancer Research. 2013;15:202.
59. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. Cell.1996;86:353–364.
60. Baeriswyl V, Christofori G. The angiogenic switch in carcinogenesis. Semin. Cancer Biol. 2009;19:329–337.
61. Jones RA, et al. Matrix changes induced by transglutaminase 2 lead toinhibition of angiogenesis and tumour growth. Cell Death and Differentiation. 2006;13:1442–1453.
62. Korner G, Schneider DE, Purdon MA, Bjornsson TD. Bovine aorticendothelial cell transglutaminase: Enzyme characterization and regulation ofactivity. Biochem. J. 1989;262:633–641.
63. Griffin M, Casadio R, Bergamini CM. Transglutaminases: Nature's biological glues. Biochem. J. 2002;368:377-396.
64. Aeschlimann D, Thomázy V. Protein crosslinking in assembly and remodelling of extracellular matrices: The role of transglutaminases. Connect. Tissue Res. 2000;41:1-27.
65. Martinez J, Chalupowicz DG, Roush RK, Sheth A, Barsigian C. Transglutaminase-mediated processing of fibronectin by endothelial cell monolayers, Biochemistry. 1994;33:2538–2545.
66. Wang Z. et al. A novel extracellular role for tissue transglutaminasein matrix-bound VEGF-mediated angiogenesis, Cell Death and Disease. 2013;4:e808. DOI:10.1038/cddis.2013.318.
67. Srinivasan U, et al. Absence of oats toxicity in adult coeliac disease. BMJ. 1996;313:1330–1331.
68. Lundin KE, et al. Oats induced villous atrophy in coeliac disease. Gut. 2003;52:1649–1652.
69. Mehta K. High levels of transglutaminase expression in doxorubicin-resistant human breast carcinoma cells. Int. J. Cancer. 1994;58:400-406.
70. Chen JS, Agarwal N, Mehta K. Multi-drug-resistant MCF-7 breast cancer cells contain deficient intracellular calcium pools. Breast Cancer Res. Treat. 2002;71:237-247.
71. Mehta K, Fok J, Miller FR, Koul D, Sahin AA. Prognostic significance of tissue transglutaminase expression in drug-resistant and metastatic breast cancer. Clin. Cancer Res. 2004;10:8068–8076.
72. Verma A. et al. Increased expression of tissue transglutaminase in pancreatic ductal adenocarcinoma and its implications in drug resistance and metastasis. Cancer Res. 2006;66:10525–33.
73. Satpathy M. et al. Enhanced peritoneal ovarian tumour dissemination by tissue transglutaminase. Cancer Res. 2007;67:7194–202.
74. Hwang JY, et al. Clinical and biological significance of tissue transglutaminase in

ovarian carcinoma. Cancer Res. 2008;68:5849–58.
75. Fok JY, Ekmekcioglu S, Mehta K. Implications of tissue transglutaminase expression in malignant melanoma. Mol. Cancer Ther. 2006; 5:1493–503.
76. Park KS, et al. Transglutaminase 2 as a cisplatin resistance marker in non-small cell lung cancer. J. Cancer Res. Clin. Oncol. 2010;136:493–502.
77. Yuan L, et al. Transglutaminase 2 inhibitor, KCC009, disrupts fibronectin assembly in the extracellular matrix and sensitizes orthotopic glioblastomas to chemotherapy. Oncogene. 2007;26:2563–73.
78. Iacobuzio-Donahue CA, et al. Highly expressed genes in pancreatic ductal adenocarcinomas: A comprehensive characterization and comparison of the transcription profiles obtained from three major technologies. Cancer Res. 2003;63:8614–22.
79. Jiang D, et al. Identification of metastasis-associated proteins by proteomicanalysis and functional exploration of interleukin-18 inmetastasis. Proteomics. 2003;3:724–737.
80. Antonyak MA, et al. Augmentation of tissue transglutaminase expression and activation by epidermal growth factor inhibit doxorubicin-induced apoptosis in human breast cancer cells. J. Biol. Chem. 2004;279:41461-7.
81. Park K-S, et al. Depletion of nucleophosmin via transglutaminase 2 cross-linking increases drug resistance in cancer cells. Cancer Letters. 2009;274:201–207.
82. Caffarel MM, et al. Tissue transglutaminase mediates the pro-malignant effects of oncostatin M receptor over-expression in cervical squamous cell carcinoma. J. Pathol. 2013;231:168-79. DOI:10.1002/path.4222.
83. Jae-Heon Jeong, et al. Transglutaminase 2 expression predicts progression free survival in non-small cell lung cancer patients treated with epidermal growth factor receptor tyrosine kinase inhibitor. J. Korean Med. Sci. 2013;28:1005-1014.
84. Assi Jasmeet, Gunjan Srivastava, Ajay Matta, Martin C. Chang, Paul G. Walfish, Ranju Ralhan. Transglutaminase 2 over expression in tumour stroma identifies invasive ductal carcinomas of breast at high risk of recurrence. PLoS ONE. 2013;8(9):e74437. DOI:10.1371/journal.pone.0074437.
85. Pierce A, et al. Transglutaminase 2 expression in acute myeloid leukemia: Association with adhesion molecule expression and leukemic blast motility. Proteomics. 2013;13:2216–2224.
86. Verma A, Mehta K. Tissue transglutaminase-mediated chemoresistance in cancer cells. Drug Resistance Updates. 2007;10:144–151.
87. Verma A, et al. Therapeutic significance of elevated tissue transglutaminase expression in pancreatic cancer. Clin. Cancer Res. 2008;14:2476-83.
88. Odii BO. Roles of transglutaminase 2 (TG2) in development of drug resistance and metastasis by cancer cells. A PhD Thesis Submitted to Anglia Ruskin University, Cambridge, UK; 2014.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=722&id=12&aid=7274