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Review Article

Spotlight on the transglutaminase 2 gene: a focus on genomic and transcriptional aspects

Nicoletta Bianchi[1], **Simone Beninati**[2] **and Carlo M. Bergamini**[1]

[1]Department of Biomedical Sciences and Specialist Surgery, Section of Biochemistry, Molecular Biology and Medical Genetics, University of Ferrara, Via Luigi Borsari 46, 44121 Ferrara, Italy; [2]Department of Biology, University of Rome 'Tor Vergata', Rome, Italy

Correspondence: Carlo M. Bergamini (bgc@unife.it)

The type 2 isoenzyme is the most widely expressed transglutaminase in mammals displaying several intra- and extracellular activities depending on its location (protein modification, modulation of gene expression, membrane signalling and stabilization of cellular interactions with the extracellular matrix) in relation to cell death, survival and differentiation. In contrast with the appreciable knowledge about the regulation of the enzymatic activities, much less is known concerning its inducible expression, which is altered in inflammatory and neoplastic diseases. In this context, we first summarize the gene's basic features including single-nucleotide polymorphism characterization, epigenetic DNA methylation and identification of regulatory regions and of transcription factor-binding sites at the gene promoter, which could concur to direct gene expression. Further aspects related to alternative splicing events and to ncRNAs (microRNAs and lncRNAs) are involved in the modulation of its expression. Notably, this important gene displays transcriptional variants relevant for the protein's function with the occurrence of at least seven transcripts which support the synthesis of five isoforms with modified catalytic activities. The different expression of the TG2 (type 2 transglutaminase) variants might be useful for dictating the multiple biological features of the protein and their alterations in pathology, as well as from a therapeutic perspective.

Introduction

Over the years, the enzymes of the transglutaminase family have been the object of extensive investigation, so elucidating their structure and catalytic activities, tissular and cellular distribution and relevance to cell biology and disease [1–4]. One particular isoenzyme, type 2 transglutaminase (TG2), is emerging as a multifunctional enzyme capable of promoting either cell growth/differentiation or cell death [5,6] under regulation by allosteric effectors. These are chiefly activities of protein transamidation, GTP-linked signalling and scaffolding of the extracellular matrix (ECM). Calcium ions trigger activation of transamidation by a conformational change shifting away the C-terminal from the N-terminal domains through a flexible connecting loop so widening the groove to free the access of the protein substrates to the active site. This process is amplified in the presence of substrates [7]. The mechanism of TG2 activation by 'opening' the structure through the domain shift is counteracted by GTP, an allosteric inhibitor that binds at the surface of domains 1 and 2 on the amino-terminal side and of domain 3, taking contact with R580 tethering the protein in the closed conformation [2,8]. In this state, the G-protein signalling activity is apparently switched-on. Data of *in situ* activity in permeabilized cells [9] and FRET technology in cultured cells support this model [10].

The expression of TG2 is likely controlled at the transcriptional level, depending on complex signalling cascades. Upon synthesis, the protein is largely present in the cytoplasm, but also in the plasma membrane and the extracellular space. A fraction of the protein is also stored in the nucleus where it participates in the control of its own expression by regulating activity of transcriptional factors to influence the promoter function [11].

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As described recently, the enzyme also presents splicing variants displaying altered regulatory properties and thereby catalytic activities [12]. The different responses of the full-length native protein to calcium and GTP (that, respectively, activate and inhibit the transamidation reaction, as mentioned) are blunted in variants with shortened sequences following splicing of the mRNA to yield, instead of the 687 AA sequence of the intact enzyme, C-terminal truncated proteins of 675, 646, 548 and 349 AA, called, respectively, *tTGv1* (tissue transglutaminase alternative splicing variant 1), *tTGv2* (tissue transglutaminase alternative splicing variant 2), TGH and TGH2 (Figure 1). The variants display reduced transamidating activity and lack functional GTP-binding sites because they lose amino acid sequences at the C-terminal region and are less sensitive to inhibition by GTP [12]. They are involved in functional Ca^{2+}-dependent activities (such as incorporation of amines into proteins and site-specific deamidation, cross-linking of proteins and isopeptide hydrolysis) also on the external cell surface for matrix assembly and cell interactions with integrins and fibronectin (FN), or Ca^{2+}-independent signal activities [3,4,13]. As a consequence of both effects, the above TG2 variants display deregulated activity in the intracellular space [14] and probably 'tonic' transamidation activity because of the predominant role of the desensitization to GTP. In addition to its roles in the intracellular compartments, an appreciable fraction of TG2 is exported to the extracellular space where it can, on the one hand, regulate the assembly and stabilize the ECM by scaffolding and protein modifying activity and, on the other hand, contribute to the transfer of extracellular signals mediated by integrins [2,15]. The mechanism of TG2 export and the eventual presence of variants in the cellular environment remain unexplained.

During the following discussion, we will identify and refer throughout to each variant as detailed in Figure 1, in which we summarize and present schematically information on the structure of the transglutaminase 2 gene (*TGM2*) gene and of the derived TG2 proteins. Figure 1 presents the organization in domains of the known

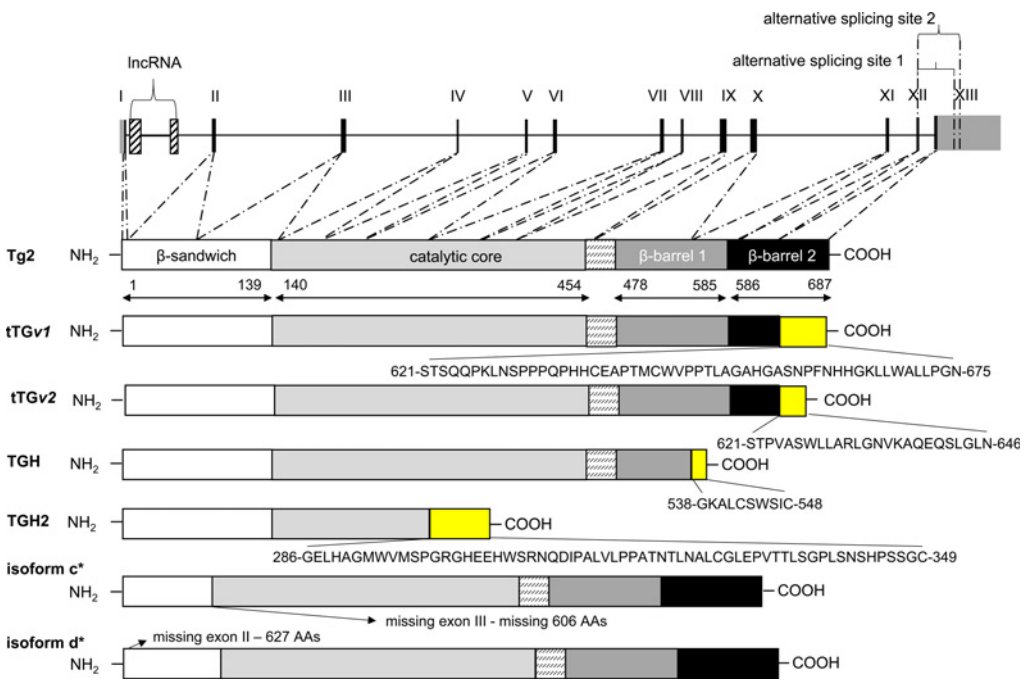

Figure 1. Type 2 transglutaminase gene and coding isoforms.

Top, *TGM2* gene is reported using roman numerals for the exons (black, coding sequences and grey boxes, untranslated sequences); furthermore, an lncRNA is indicated within the first intron, composed by two exons (slanting line boxes); the position of alternative splicing sites producing *tTGv1* and *tTGv2* isoforms is also indicated. The correspondence between exon structure and protein domains is evidenced by the dotted line. The four structural domains of TG2 have been painted as follows: white, β-sandwich; two shades of light grey, catalytic core and β-barrel 1; black, β-barrel 2; dashed box, the loop. The limit AA in each domain is bordered by arrows. Altered isoforms *tTGv1*, *tTGv2*, TGH and TGH2 are reported; in yellow, the modified tails of these truncated isoforms. Bottom, two putative isoforms are included (c* and d*); as reported in the following web site https://www.ncbi.nlm.nih.gov/nuccore/NC_000020.11?report=genbank&from=38127387&to=38166578&strand=true.

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isoforms of TG2, which are generated by differential splicing events, while Figure 2 reports by means of distinct stains the main protein's functional regions, as extensively described in the literature [4,6,16,17].

The molecular biology of the *TGM2* gene is much less understood than the biochemical and cell biology issues of the enzyme, despite a long-standing knowledge of *TGM2* and the recent detection of single-nucleotide polymorphism (SNP) [18] and of protein variants derived from alternative splicing [12]. After a brief survey of the basic knowledge of the TG2 protein, we now scrutinize these rather unexplored issues of *TGM2*, reviewing data emerging from scientific literature and genomic databanks. Our investigation revealed several intriguing issues, leading to the conclusion that, like the protein, the gene can still yield many surprises. *TGM2* is a large 40 kb gene transcribed into a 5 kb primary RNA sequence containing 13 exons and 12 introns, further converted into a mature 2 kb mRNA. Untranslated regulatory regions are present and increase the complexity of this gene which is under the control of a promoter sensitive to multiple transcriptional factors, and to vitamin, hormone and environment responsive elements. Although comparatively neglected when set beside TG2's biochemistry and cell biology, *TGM2's* molecular biology has also accumulated a large amount of information to draw on. As readers will easily appreciate in our presentation, we wish to focus the attention on the *TGM2* gene, its transcription products and on interfering non-coding molecules. We have divided the presentation into the topics: (i) TG2 functions in relationship to its cellular location; (ii) gene structure; (iii) gene regulatory sites and binding of transcription factors; (iv) transcriptional variants and (v) the roles of regulatory RNAs.

Selective roles of TG2 in different cellular locations

While the roles of TG2 in the cytosol (reviewed in refs [2,3]) are mainly related to protein modification at glutamine residues (transamidation, leading to cross-linkage or post-translational modification of substrate proteins by primary amines or hydrolysis of glutamine amide moieties), the additional functions of signal transduction (as a member of the G-protein family) and of protein scaffolding for the correct assembly of the ECM are chiefly brought about by the protein present at the membrane level. It is currently well known that translocation of TG2 to different compartments (including also nucleus and mitochondria beyond the plasma

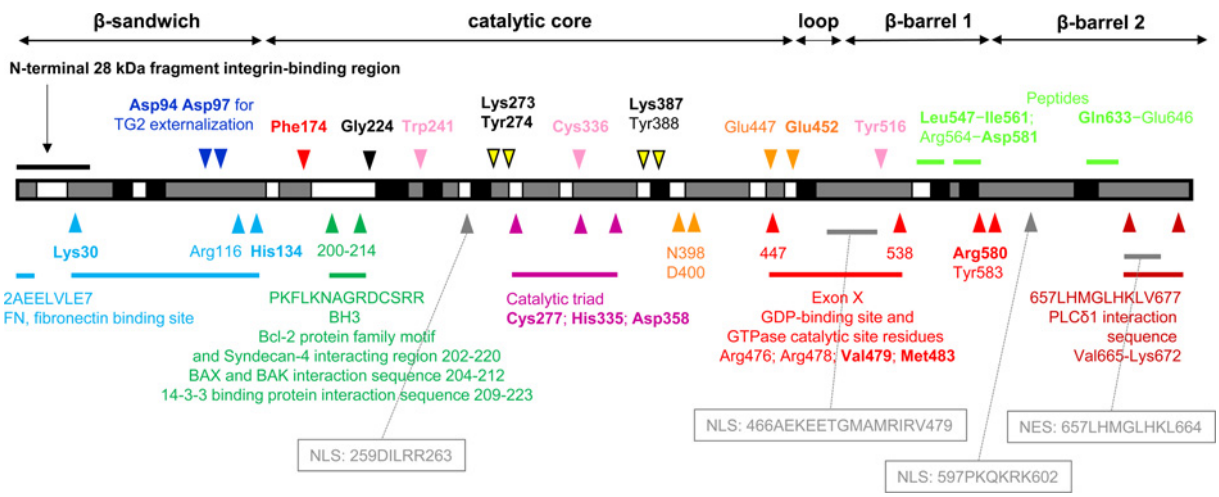

Figure 2. Functional regions and key amino acids.

The domains (β-sandwich, catalytic core, β-barrels 1 and 2, and the loop) are related to the secondary structure of TG2 depicted with a barrel, where white indicates helix, black turn and grey β-strand (from the Uniprot web site: http://www.uniprot.org/uniprot/P21980). Along this structure, we indicate with different colours of functional regions and key amino acids: cyan, FN-binding site and residues L30, R116 and H134 of TG2, which are crucial to interact with FN; black bar, integrin-binding region and blue arrows, residues involved in TG2 externalization. Dark green, Bcl-2 protein family motif; red, exon X containing key residues forming GDP-binding and GTPase sites, and Phe174 red arrowed; lilac, catalytic triad (C277, H335, D358); pink, W241, C336 and Y516 residues engaged in transamidation activity, stabilization of transition state and inhibiting disulfide activity through the C277 binding; orange, putative Ca^{2+}-binding sites predicted from the FXIIIa structure; black, triangle evidence G224, which modulates Ca^{2+}-binding affinity; yellow, AAs in non-proline *cis* peptide bonds; brown, domain recognizing PLCδ1; grey, NLS and NES; brilliant green, three peptide regions involved in the activation of TG2 by the $\alpha 1^B$-adrenergic receptor (L547–I561, R564–D581 and Q633–E646). Bold, residues without SNPs reported for that codon.

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membrane) is associated with specialized enzyme functions. Thus, the extracellular form promotes cell survival and control of cell–protein interactions in the extracellular space for tissue stabilization and angiogenesis, while in the intracellular space (in the cytoplasm, but also in the nucleus and membranes) the role of the protein is more likely linked to cell death and differentiation [19]. In these processes, signals inside and outside the cells possibly cross-talk reciprocally in modulated interconnected ways.

These somewhat divergent functions depend on stabilization of distinct conformational states by interaction with the ligand calcium and GTP or by changes in the cell redox state (leading to protein disulfide exchange) [20] that act in concert to keep silent the transamidation reaction favouring the signalling activity under physiological conditions, while the reverse is true in stressful conditions.

Taking into account peculiarities of TG2 activities in different locations, it appears that the fraction of TG2 bound to membranes can be involved both in protein cross-linkage and signalling. In particular, it modifies (i) glutaminyl substrate proteins to improve stability and flexibility on the tissue structure organization or modify laminin and nidogen to membrane stabilization, and integrins/heparan sulfate chains of syndecan-4 (SDC4) interfering in cell adhesion and migration [21], as TG2 interacts with integrins in the ECM. This occurs through high-affinity binding between the gelatin-binding domain of FN and the TG2 N-terminal domain; again, the second C-terminal β-barrel domain of TG2 could bind other ECM partners implicated in human pathology, as in coeliac disease [22]. In the extracellular space, TG2 and FXIIIA are engaged in the formation and stabilization of initial complexes between FN and Collagen I for matrix production during differentiation and mineralization of human cultured osteoblasts [23], but also contribute in vessels to the stabilization of fibrin clots and of atherosclerotic plaques [24].

Since it is known that TG2 has no obvious secretory exocytosis-targeting signal sequence, it is probably externalized by unconventional secretion mechanisms, in which specialized cotransport complexes or features (like post-translational modifications, secondary structure, abundance of charged residues, propeptides and other transmembrane helical regions) could contribute to cross the plasma membrane. Alternatively, some kinds of vesicles could internalize cellular TG2 and secrete it outside by exocytosis in response to external stimuli. A recent study identified TG2 in vesicles of both exosomes and microvesicles/ectosomes; however, only in exosome, lysate was it observed (tubular epithelial cells and SDC4 knockout mice) [25]. Q3

Whatever the case the extracellular trafficking of TG2 seems crucial to regulating ECM homeostasis; thus, in order of defining critical domains required for TG2 export, mutated expression vectors were constructed to transfect kidney tubular epithelial cell lines [26]. Mutations of D94 and D97 within the N-terminal β-sandwich of the FN-binding domain were crucial for TG2 export, although siRNA knockdown of FN experiments produced no similar effects, so demonstrating that it was substantially FN-independent [26].

In the extracellular space, TG2 is believed to be transiently activated in a way largely dependent on oxidative effects [27] which are more relevant than inside the cells, because Ca^{2+} and GTP are present at high and low levels and are unlikely to change rapidly. Because of the oxidizing nature of the extracellular space secreted proteins often present disulfide bonds as post-translational modifications to preserve protein structure, their cleavage managing to restore enzymatic activity. In the case of TG2, this is brought about by thioredoxin, a ubiquitous thiol reductase released from cells in response to oxidative stress signals [28]. It has a conserved active site motif operating reversible oxidation through the transfer of reducing equivalents to cleave disulfide bond of C370–C371 on TG2, because the oxidation can interfere with Ca^{2+} activation. These structural changes in the protein's architecture also involve C230, which plays a role as a redox sensor [3]. Furthermore, in the extracellular environment, S-nitrosylated TG2 could provide a reservoir of NO bioactivity producing anti-inflammatory features at the endothelial cell surface [3]. S-nitrosylation at active site C277 inhibiting TG2 seems to regulate its externalization.

On the inner surface of the plasma membrane, notably TG2 is implicated in transmembrane signalling through interactions with the oxytocin receptor, thromboxane A2 receptor α isoform, $\alpha1^{B}$- and $\alpha1^{D}$-adrenoreceptors and phospholipase C δ1 (PLCδ1), acting like a G-protein. In this context, the GDP/GTP-bound TG2 has not a transamidating activity, but transduction signalling one, reversed by Ca^{2+}.

Additional compartments to which TG2 is associated are mitochondria, endoplasmic reticulum and nucleus. Both mitochondria and endoplasmic reticulum represent compartments for Ca^{2+} storage and their membranes prove to be interconnected by mitochondria-associated membrane proteins. In this location, TG2 plays a role in regulating calcium homeostasis, e.g. by cross-linking inositol 1,4,5-triphosphate receptors, formed by allosteric subunits assembled into a calcium channel, locking it in an irreversible manner [29]. On the other hand,

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the enzyme could act on Ca^{2+} homeostasis, also modifying voltage-dependent anion-selective channels upon glucose stimulation or guanine exchange factors through uncharacterized signalling pathways [29].

TG2 present in mitochondria drives apoptosis. Most of the enzyme is associated with the outer membrane and the intermembrane microenvironment, while only a few are associated with the inner mitochondrial membrane and the mitochondrial matrix [29]. TG2 contains the 204LKNAGRDC211 sequence highly homologous to the BH3 domain of Bcl-2 family proteins, where L204 is crucial for induction of hyperpolarization of the mitochondrial membrane. This peptide sequence interacts with proapoptotic Bax, but not with antiapoptotic Bcl-2, and thus, the proapoptotic or antiapoptotic effects might to be opposed and depend on cell type and cell death inducer [3]. In mitochondria, TG2 catalyzes transamidation of proteins leading to covalent modification of mitochondrial proteins associated with pathological conditions. This occurs, for instance, in cardiac ischaemia and reperfusion injury in the heart of $TG^{-/-}$ mice with phenotype similar to maturity-onset diabetes of the young (MODY), or in Huntington's disease (HD), where it causes also heavy effects on energy metabolism under stress conditions interacting with metabolic enzymes [29]. In addition, in the mitochondria, alteration of TG2 disulfide isomerase activity targeting oxidoreductases, cytochrome *c* oxidase and ATP synthase, produces strong effects on mitochondrial physiology [30].

The features that direct TG2 to a nuclear translocation are not yet completely characterized. This ability has been ascribed to two sequences in the protein, 259DILRR263 and 597PKQKRK602 [31]. Recently, a study on hepatocellular carcinoma cells demonstrated nuclear accumulation of TG2 after treatment with acyclic retinoids [32], which increase TG2 trimeric complex with importin-α and importin-β and identify two new important signal, one is nuclear location signal (NLS), the sequence 466AEKEETGMAMRIRV479 in the β-barrel 1, and the second is a leucine-rich nuclear export signal (NES), the sequence 657LHMGLHKL664 in the β-barrel 2.

Upon its translocation to the nucleus, TG2 can modify and inactivate transcription factors, including Sp1 leading to reduce expression of growth factor receptors, such as c-Met and EGF, involved in cell survival [3]. Only ∼5% of the total TG2 is found in nucleus, where it can interact with chromatin-modifying histones H3, H2A, H2B and H4, altering specific gene expression [3]. Especially the polyamination of H3 appears to correlate with decreased transcriptional activity of several genes, e.g. peroxisome proliferator-activated receptor-γ, co-activator 1α affecting cytochrome *c* levels and the expression of other genes involved in biogenesis and function of mitochondria [4,29].

Otherwise, there are instances reporting transcriptional coregulatory effects of TG2 [3], mediated by the direct non-covalent binding of TG2 to transcription regulative factors independently of the enzymatic activity. This is the case of the interaction with c-Jun inhibiting c-Jun/c-Fos dimerization, blocking interaction at Ap1 site in the MMP-9 gene promoter in cardiomyoblasts. Analogously, in cortical neurones, TG2 affects HIF-1β/HIF-1α dimerization and genes containing hypoxia-response element (HRE). Finally, protein kinase activity of TG2 is suggested to phosphorylate H1 and H3 histones, p53 and retinoblastoma protein (Rb). The phosphorylation of Rb by TG2, inhibited in the presence of high Ca^{2+} levels, destabilizes the Rb/E2F1 complex, while it seems to enhance when protein kinase A phosphorylates TG2 [33].

Owing to the polyhedric nature of this enzyme, many aspects of its nuclear functions must be further investigated.

The DNA face of human *TGM2*: SNPs in sequences coding amino acids with structural and functional roles

The sequence of human and murine TG2 [34] and the organization of the *TGM2* gene [35] were reported years ago and then further refined. The human gene is 40 kb long in chromosome 20 with a transcribed region involving 13 exons and 12 introns. The CAAT and TATA sequences, the +1 transcription starting site and the binding sites for several transcription factors (including Sp1, Ap2 and NF1) have been defined in a 2 kb promoter region with regulatory elements upstream of the ATG translation start codon [36]. Only ∼5 kb constitute RNA transcript whose 13 exons are translated into the canonical TG2 protein containing 687 AAs. The gene includes also untranslated 5′ and 3′ regions which can be involved in the generation of transcription variants and a long non-coding (lncRNA) molecule in the first intron, something we will discuss in detail in the following sections.

TGM2 displays an appreciable degree of SNPs investigated by Király et al. [18] in relation to altered functions of modified isoforms using information from the 1000 Genomes Project Consortium. A large number of SNPs are indeed available in net databases, including HeapMap, Ensemble and NCBI. The NCBI database

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(https://www.ncbi.nlm.nih.gov/) describes more than 3000 SNPs in the *TGM2* gene, but notably only 293 of them produce non-synonymous residue substitution, frameshift or insertion of a stop codon that can affect the amino acid sequence. Sixty-seven SNPs occur in the β-sandwich region of the protein, 135 in the catalytic core, 8 in the loop structure, 34 in β-barrel 1 and 49 in β-barrel 2. The SNPs reported in Supplementary Table S1 along with their allelic frequency are mostly missense SNPs and concern codons, which could determine functional consequences in TG2 structure, due to their location in putative critical regions further documented in Figure 2. We underline that only six SNPs occur in homozygous status: the highest number is 5 people for the SNP causing R76H substitution (within the FN-binding region), 3 people with V542F modification (beyond GDP-binding region), while only one subject reported the substitutions R222Q (near to G224, modulating Ca^{2+}-binding affinity), R433Q (near the GTPase catalytic region), P612T (between one NLS and the peptide-binding region) and D671N (PLCδ1 interaction sequence), a matter we will discuss in further detail below.

Supplementary Table S1 also lists the SNPs involved in alterations at 'amino acid clusters' of TG2 that were recently investigated by Thangaraju et al. [37]. These authors focused attention on short sequences of 3–4 amino acids differing in murine and human TG2, which could be engaged in functional activity or stability of the protein, in the interaction with other proteins and in target sites for post-translational modifications. These short peptides contain amino acids that might influence FN-binding (Q15 located within the sequence L14-T16), or regulation by Ca^{2+} (Q324 and G325). Other important residues could be L657 and H658, which are involved in binding interacting proteins, and are neighbours to M659. This amino acid belongs to the conformational epitope 2 recognized by auto-antibodies of coeliac disease [38], along with R19, for which two putative SNPs are reported. The NCBI database identifies in coeliac epitope 2 an additional SNP at E154, while only one SNP affects epitope 1 with the R116C substitution [39]. Several SNPs in Supplementary Table S1 target amino acids undergoing possible post-translational modifications, such as rs370982887 that abolishes phosphorylation of S60 (Uniprot web site, http://www.uniprot.org/uniprot/P21980) causing S60G substitution [40] and rs754225495 that produces a stop codon at E467 included in the region of GDP-binding/GTPase catalytic sites and could also prevent N-acetylation of K468 (occurring in mice).

In Figure 3, we summarize additional SNPs coding mutants at structural and functional domains of the Q6 protein in natural or artificial variants produced by site-specific mutagenesis [18], which are of particular interest for their association with relevant structural changes. Two SNPs (one identified in the natural variant VAR_052553 causing R76H substitution and the other Q324R in VAR_055358) affect only marginally interaction with FN tested by ELISA using monoclonal antibodies or catalytic activity in the FN-bound form, while the substitution R214H leads to marked loss of transglutaminase activity. This SNP, responsible for the VAR_055357, is located in the sequence amino acids 200–223 AAs involved in protein binding [41]. The natural variants VAR_055359 and VAR_052554 (harbouring the replacements D389N and R436W) have very low transglutaminase activity; this increases, however, in the enzyme stabilized by binding to FN, in analogy with substitution G660V (occurring in natural variant VAR_036554) described as a somatic mutation in a patient affected by colorectal cancer [42]. Király et al. [18] studied variants of TG2 associated with exonic specific SNPs by activity assay and ELISA using antiserum from untreated coeliac patients. They proved that the mutant G660V interacts with the antiserum with lower affinity than R214H (VAR_055357, above reported) and P536S (SNP at the GTP site in variant VAR_052554). These data are conceivable if mutant G660V was involved directly in the interactions of TG2 with coeliac disease auto-antibodies, likely due to proximity with the coeliac epitope residue M659 [38]. The already mentioned SNP in R116 at coeliac epitope 1 interested with the FN-binding site has not been associated with any known kind of natural variant [39].

In Figure 3, we focus our attention (Figure 3B) on the algorithms TANGO, WALTZ and FoldX (ddG) to analyze the given mutations (indicated with a star in Figure 3A): TANGO is based on physico-chemical principles of secondary structure formation, to predict prone regions; WALTZ makes it possible to identify amyloid-forming regions in functional amyloids; ddG by FoldX is the difference in folding free energy change between wild-type and mutant protein to predict absolute values of protein stability.

The dTANGO value is significant only for the mutation M330R, while positive values of the difference in free energy of the mutation (ddG) that produce a destabilizing structure when in the range between +1 and Q7 +5 kcal/mol were detected for the mutations M330R and I331N described in subjects of with early-onset type 2 diabetes [43], or for the somatic mutation G660V [42] and the substitutions R214H and P536S investigated by Király et al. [18]. A negative dWALTZ score of hexapeptide used to predict amyloidogenicity is reported only for the M330R and I331N mutations corresponding to a decreased amylogenic tendency. Finally, as listed in Table 1, these natural variants are associated with amino acid modification in functional domains, as well as

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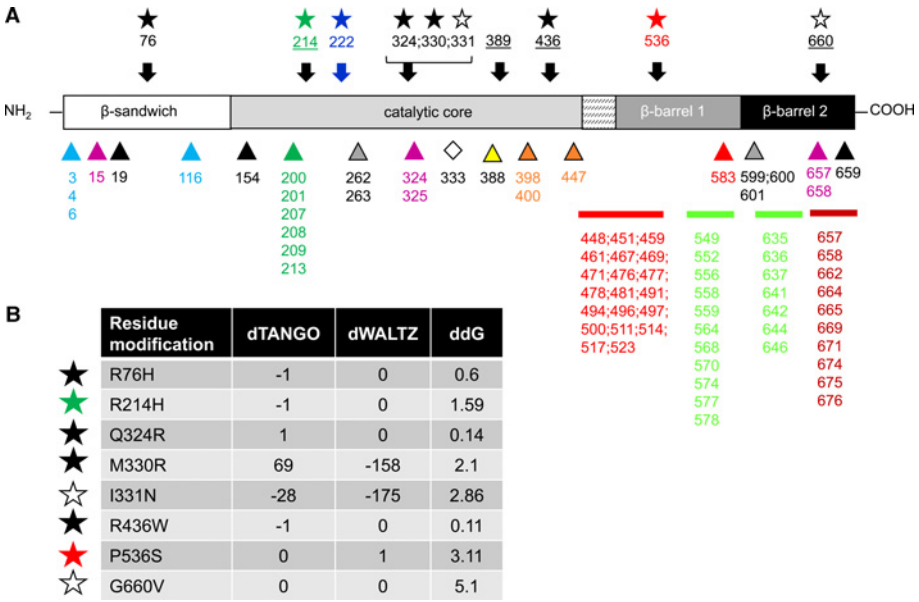

B

	Residue modification	dTANGO	dWALTZ	ddG
★	R76H	-1	0	0.6
★	R214H	-1	0	1.59
★	Q324R	1	0	0.14
★	M330R	69	-158	2.1
☆	I331N	-28	-175	2.86
★	R436W	-1	0	0.11
★	P536S	0	1	3.11
☆	G660V	0	0	5.1

Figure 3. Transglutaminase 2 variants and SNPs in the structural domains.

(**A**) Stars represent natural variants of the protein linked to SNP and described in the Uniprot database, where white stars indicate natural variants associated with the mutations I331N and G660V reported in the literature [42,43], while other stained stars have the same colour code as reported below. The variants identified by black arrows have been cloned and tested by Király et al. [18], who reported for some of them (underlined) loss of transglutaminase activity in the enzyme bound to FN, whereas that by blue arrow is associated with phenotype with the evidence of functional modification [44]. The white rhombus represents mutation N333S in a subject with MODY [43,45]. Triangles and bars represent SNPs detected in functional regions: cyan, SNPs in FN-binding site; violet, SNPs in amino acids probably significant for human *TGM2* activities as described by Thangaraju et al. [37]; black, SNPs in epitopes interacting with coeliac antibodies; green, SNPs in Bcl-2 protein family motif; grey, SNPs in nuclear location signals; yellow, SNP in amino acid in non-proline *cis* peptide bonds; orange, SNPs in putative Ca^{2+}-binding sites; red, SNPs in GDP-binding and GTPase sites; brilliant green, SNPs in peptide regions involved in recognition and activation by the $\alpha 1^{B}$-adrenergic receptor; brown, SNPs in domain for PLCδ1 recognition. (**B**) Effects of SNP on the variant phenotype from the web site (http://snpeffect.switchlab.org/menu). In the table, we present a summary of the algorithms dTANGO, dWALTZ and FoldX (ddG) scores used to predict stability of the natural TG2 variants indicated by stars in (**A**). dTANGO score outside the range of −50 to +50 is considered significant between wild-type and mutant protein; dWALTZ score combines sequence, physico-chemical as well as structural information into a composite scoring function, in which positive peaks indicate an increase and negative peaks indicate a decrease of amylogenic tendency due to the variant; FoldX is used to calculate the difference in free energy of the mutation, no effect on stability for ddG value between −0.5 and +0.5 kcal/mol, while positive values indicate a reduction of the stability (slightly between +0.5 and +1, severe when higher than +5 kcal/mol); conversely, negative value indicates enhanced stability (slightly between −0.5 and −1 kcal/mol, greatly enhanced stability lower than −5 kcal/mol).

their predicted enzyme stability (as desumed from the web site http://snpeffect.switchlab.org/uniprot/TGM2_HUMAN?order=Disease&sort=asc), and the frequency of the minor allele. The rs41274720, coding R76H substitution, is the only SNP reported in homozygous status and presents the highest frequency, possibly for its low effects on the stability of the protein, considering how little represented are mutations affecting the conserved structure of this enzyme.

Only a few (29/272) non-synonymous single-nucleotide variants (nsSNV) in *TGM2* gene [44] can produce loss of function, when analyzed using the database ExAC (Exome Aggregation Consortium browser) and the scores Sorting Intolerant From Tolerant (SIFT) and Polymorphism Phenotyping (PolyPhen). The authors investigated both the effects of the rare SNVs on the conserved sites involved in important functional roles and the sites or motifs interested with protein–protein interactions focusing the attention on homozygotes-related nsSNVs. Six variants with an allele frequency of <0.5% (R76H; R222Q; R433Q; V542F; P612T; D671N) were

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Table 1 TG2 natural variants described as the target of SNP and in the literature reported

These natural variants are reported in the literature or taken from the web site http://www.uniprot.org/uniprot/P21980. For each of them is defined SNP sequence number identification (https://www.ncbi.nlm.nih.gov/snp) and references, amino acid substitution, functions or involved domains, finally reference number variant. Number of homozygotes was obtained from http://exac.broadinstitute.org/, while the minor allele frequency/minor allele count was obtained from both http://exac.broadinstitute.org/ and https://www.ncbi.nlm.nih.gov/snp or https://genome-euro.ucsc.edu, and the SNP effects are predicted from the web site: http://snpeffect.switchlab.org/uniprot/TGM2_HUMAN?order=Disease&sort=asc.

dbSNP rs# cluster id/ reference	Amino acid residue modification	Function/involved domain	Natural variant	Number of homozygotes	Minor allele frequency	Predicted effect on stability
rs41274720 [18,44]	R76H	Non-synonymous single-nucleotide variants (nsSNVs) associated with homozygotes in 12 individuals	VAR_052553	5	4.798×10^{-3}	Slightly reduced stability
rs45530133 [18,44]	R214H	BH3 Bcl-2 protein family motif Damaging nsSNVs in amino acid sequence of *TGM2* by PolyPhen/SIFT scores. It had destabilizing ΔΔG values in the opened and closed conformations Syndecan-4 interacting region; 14-3-3-binding protein interaction sequence	VAR_055357		4.365×10^{-5}e	Reduced stability
rs45567334 [18,37]	Q324R	I323-D326 Might influence Ca^{2+} regulation	VAR_055358		5.263×10^{-5}	No effect on stability
rs141603506 [43]	M330R	Described in patients with early-onset diabetes type 2; (pathological significance unknown)	VAR_037998		1.730×10^{-4}	Reduced stability
[43]	I331N	Described in patients with early-onset diabetes type 2 (pathological significance unknown)	VAR_037999		n.d.	Reduced stability
rs45629036 [18]	R436W		VAR_055359		7.827×10^{-4}	No effect on stability
rs45556333 [18]	P536S	GDP-binding and GTPase catalytic sites	VAR_052554		8.74×10^{-4}	Reduced stability
[42]	G660V	Described in a colorectal cancer sample, like a somatic mutation	VAR_036554		n.d.	Severely reduced stability

produced *in vitro* using the site-directed mutagenesis strategy, since they could damage the secondary structure of TG2 and its function. These variants were tested in biochemical assays, but the majority of them did not cause major changes in activity. Only the variant R222Q demonstrated lower amine incorporation into substrates at 0.25 mM Ca^{2+} concentrations, while full activity was recovered at higher concentration of the cation. This substitution probably affects TG2 conformation because it is located near G224, which modulates Ca^{2+}-binding affinity (Figure 3, blue arrowed).

To date, association between the occurrence of SNP in the *TGM2* and disease has been explored in only a few clinical studies and the relevance of these associations is still poorly defined. In Figure 3, we collect the amino acid substitutions related to a characterized SNP in the sequenced genomes, referred in the literature. The missense mutations involving M330R (VAR_037998) and I331N (VAR_037999) that were described in subjects of with early-onset type 2 diabetes and not detected in 300 normoglycaemic controls should be further checked to assess pathological significance [43]. A nearby missense mutation N333S (marked by a white lozenge) was detected in heterozygous status in an Italian patient with MODY [45]. The same mutation was detected in the genome of the father, a diabetic like other family members not analyzed. These three modifications in the amino acid sequence of TG2 are located between the transamidation catalytic site and the GTPase domain in regions that are conserved in the genome throughout evolution; particularly, N333 is constant in all members of transglutaminase family and could contribute to the complex phenotype causing hyperglycaemia.

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particularly, N333 is constant in all members of transglutaminase family and could contribute to the complex phenotype causing hyperglycaemia. When these mutations were cloned into the *TGM2* expression vector and transfected in human COS7 cells, they impaired transamidating activity (which was reduced to 33.1 ± 4.1 nmol/h/mg of protein for I331N, 75.5 ± 8.5 nmol/h/mg for M330R and 81.9 ± 10.2 nmol/h/mg for N333S, when compared with wild-type control values of 165.4 ± 13.4 nmol/h/mg) [43]. Among these, N333S mutation could be the most relevant, although clearly heterozygous *TGM2* mutations are not the only factor causing diabetes in the investigated families.

The analysis of their effects on TG2/*TGM2* function has been checked in TG2 knockout mice, which are very sensitive to insulin in glucose tolerance test [45]. Other results suggest a relationship between their hyperglycaemic status and the mechanism of secretion of insulin granules [46]. In contrast with support a direct association between TG2 alteration and glucose homeostasis, there are date on TG2 knockout mice reported by Iismaa et al. [47], who did not detect any link, although we cannot exclude species-specific aspects or interferences by other pathways on insulin secretion.

Another relevant region of interested to SNPs is that spanning exons V and VI of *TGM2* (Supplementary Table S1) which are targeted to produce TG2 knockout mice. Deletion of this sequence modified phagocytosis of apoptotic cells by macrophages, possibly giving rise to autoimmune reactions [48]. Most SNPs of *TGM2* gene reported in subjects with pathologies occur within intronic sequences. Bradford et al. [49] genotyped eight SNPs of *TGM2* gene representing intron variants associated with schizophrenia in a British cohort of patients; this suggests the relevance of the SNPs rs4811528, rs2076380, rs6023526 and rs7270785 in the modulation of *TGM2* expression. Further studies demonstrated that rs4811528 SNP is also associated with raised levels of interleukin 2 (IL-2) in plasma, but without sufficient evidence to define this condition as a predisposing factor to develop schizophrenia [50].

The relative low number of SNPs in the coding region of this protein suggests an evolutionary strong selection on the structure needed to conserve its functional features [18,44,51]. As a consequence, it could be possible that a lot of missense mutations would not be selected during evolution and finally from the feasible genotypes in the population.

However, some nsSNVs for *TGM2* occur in cancer cells without affecting the genome in other districts, as resumed in the integrated BioMuta dataset (https://hive.biochemistry.gwu.edu/cgi-bin/prd/biomuta/servlet.cgi?gpageid=11&searchfield1=gene_name&searchvalue1=TGM2), where frequency of variation is referred to each cancer. A higher frequency of *TGM2* variations was found in colon, skin, stomach, uterus, lung, liver, breast, ovarian and head–neck cancers, respectively, and the more frequently amino acidic positions interested to nsSNVs are R476 mutated to Thr (frequency 12) in GDP-binding/GTPase catalytic site and A207, R296, R317, K364, R377, R512 (frequency 10) with variable amino acid replacement. No specific reason for these frequent variations has been combed.

The DNA face of human *TGM2*: binding sites for transcription factors in upstream regulatory regions

Information is now available on the regulation of *TGM2* expression, through binding of transcription factors at the gene promoter or any other regulatory region. The promoter, originally described by Lu et al. [36], involved a sequence of 1.74 kb in the upstream region of *TGM2*, containing TATA and CAAT boxes, hormone responsive elements and some putative binding sites for transcriptional factors. Cloning the region in a luciferase reporter vector, the authors demonstrated its promoter functions in transfected cells. They also produced fragments deleted of the glucocorticoid and IL-6 receptors (named dTdG-rich region) and Ap2-responsive elements defining a 'core' promoter that allowed identification of four Sp1 sites (−56/−51; −45/−40; +57/+68) contributing to the minimal promoter (as in Figure 4), whereas the presence of NF1 sites was not so relevant for the transcriptional activity. One mutant was also constructed to test the transcriptional induction of *TGM2* by retinoids in responsive cell lines, independently of any modulation by sequences in this 5′-UTR analyzed fragment.

Subsequent studies [52] yielded information on the methylation of the promoter in normal and neoplastic human cells. Two CpG-rich regions (at −205/+75 and at −1415/−1315) were recognized sites of DNA methylation in the 1.74 kb promoter fragment with correlation between the degree of methylation and constitutive transcriptional activity in HUVEC cells and human erythroleukemia K562 cells (having a high level of basal transglutaminase activity) or monocytes, lymphocytes and HL-60 cells (presenting low enzyme activity).

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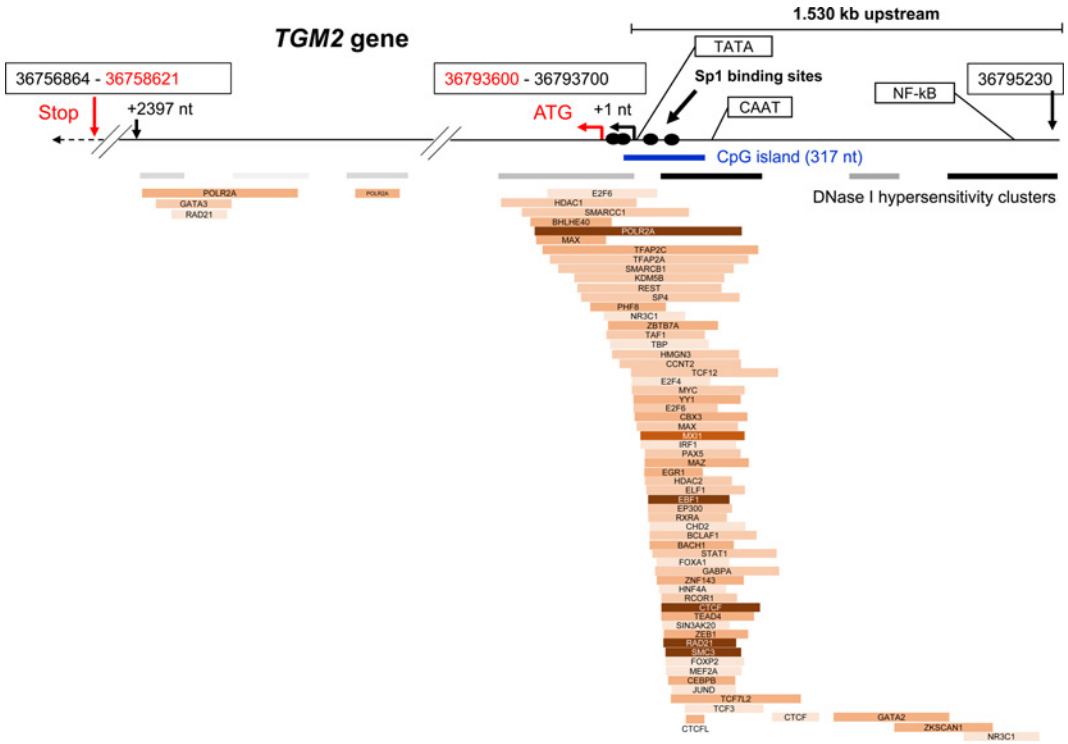

Figure 4. 5′-UTR region of *TGM2* containing transcriptional regulatory elements and transcription factor-binding sites.
Approximately 1530 bp of *TGM2* promoter are detailed. In red, start and stop codons of translation, whereas in black, start and stop of transcription from Human GRCh37/hg19 Assembly in the Genome Browser on chromosome 20. CAAT, TATA, Sp1 and NF-κB-binding sites are represented as in the literature. Blue, CpG island; black and grey shade boxes, DNase I hypersensitivity clusters; shades of brown, tracks derived from transcription factor ChIP-seq of Genome Browser analysis, where darker colour corresponds to the major intensity of transcription factor binding to consensus sequence.

Further results indicated possible association between hypomethylation of the promoter and increased levels of TG2 activity upon suppression by treatment with 5-azacytidine. The role of hypermethylated 5′-flanking regions of *TGM2* has been demonstrated also in epigenetically silenced human glioma cells [53]. Studies on CpG methylation of the first island proximal to translation starting site by pyrosequencing after bisulfite conversion in glioma cell lines and in tumour or normal brain tissues demonstrated association between *TGM2* methylation state and cancer, with *TGM2* overexpression in the more aggressive neoplasia.

Representative schemes of the *TGM2* promoter and of its regulation are available [54,55], pointing towards key roles of inducers as controllers of the rate of *TGM2* transcription and chief regulators of the cellular levels of TG2. Of major physiological relevance are the effects of promoter binding by NF-κB and retinoid receptors which both are powerful inducers of expression. Binding of NF-κB (as schematized in Figure 4) strongly stimulates enzyme induction [56] by different mechanisms linked together and consisting of a direct effect of NF-κB on the promoter and of uncanonical activation of NF-κB by TG2-mediated polymerization of the regulatory protein IκBα (inhibitor of nuclear factor-κBα) [57] in a positive feedforward fashion, as detailed later. Analogous effects of TG2 on the regulation of activity of transcription factors by protein polymerization have been described for HIF-1α and for von Hippel Lindau protein. Regarding regulation by retinoids, *TGM2* promoter presents RARE sequences, which mediate induction of *TGM2* by RXR and RAR ligands, as originally investigated in Davies' laboratory and tentatively linked to activation of the RXL receptor pathway in cultured phagocytic cells [58]. Recently, further studies on the genome architectural context of RXR-regulated genes [55] defined active RXR enhancers and the mechanisms of induction of transcription. Combining advanced techniques of nucleic acid analysis (RNA-seq, RT-qPCR, ChIP-seq, GRO-seq and 3C-seq, i.e. chromosome conformation capture and sequencing), Daniel et al. [55] detected in murine bone marrow macrophages a large number of retinoid-sensitive genes; they noted that *TGM2* responded to three typical ligand RXR regulatory

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units. The authors resolved the differential assembly of transcriptional repressor CTCF and RAD21, a member of the cohesin complex, in the absence or presence of ligand-activated RXR and predicted generation of loop structures based on CTCF/RAD21-cobound regions. They observed a decrease of RAD21 binding upon RXR ligand treatment without evidence of tighter binding of RAD21 on active RXR enhancer elements, when compared with non-active sites. This CTCF/RAD21 connection could stabilize chromatin loops of the preinitiation complex, in which also enhancer RNA molecules (eRNAs) were engaged. So, RXR either as a heterodimer or potentially as a homodimer could bind to a genomic region along with PU.1 to recruit P300 and release PU.1. Finally, the preinitiation complex was assembled to start transcription.

N-Myc and c-Myc also contribute to regulate *TGM2* expression in neuroblastoma and in breast cancer cells, apparently through recruitment of histone deacetylase 1 protein (HDAC1) to the promoter ([59], but see also below). The Myc protein can both induce and repress TG2 expression depending on the formation of two distinct regulatory protein complexes: in one case, N-Myc acts as a transactivator together with Max, Tip-60 or TRRAP factors, and in the other, N-Myc acts as a repressor together with Sp1 when HDAC1 is also engaged in the complex, inhibiting the activity [59]. In neuroblastoma, N-Myc is likely involved in resistance to retinoids, occurring in over 50% of treated patients [60]. In this tumour, N-Myc represses while retinoids activate expression of the TG2 and TGH isoforms, both containing the transamidase site (with the catalytic triads C277, H335 and D358), with the inhibitory GTP site (with its R580 residue) present only in TG2. Consequently, differential expression of these variants gave rise to alternate responses matching observations that TGH induced and TG2 repressed neuroblastoma cell differentiation through GTP-binding and enhanced expression of vasoactive intestinal peptide. An overall involvement of N-Myc in controlling expression of TG2 and TGH and differentiation of neuroblastoma cells was confirmed by N-Myc silencing with siRNA. At the same time, treatment with retinoids increased both TG2 and TGH, and overexpression of TG2 reduced retinoid-induced cell differentiation. These data point towards the development of anticancer molecules, as inhibitors of GTP-binding or as activators of transamidase activity of specific isoforms.

Features of tissue-specific *TGM2* expression have been explored in only a few experimental models, in thymic lymphocytes, triggered to apoptosis by different treatments *in vivo* (retinoids, glucocorticoids, antibody activation of cell surface death receptors and DNA damage by ionizing radiations). In transgenic mice carrying a 3.8 kb fragment of the *TGM2* promoter coupled to β-galactosidase to probe TG2 expression in 4-week-old animals, dexamethasone caused thymus atrophy [61], with a rise of β-galactosidase activity, which occurs also after irradiation, anti-CD3 treatment and CD437 injection. When the same stimuli applied during *in vivo* experiments were employed to induce apoptosis *in vitro*, no endogenous TGase and β-galactosidase activities were observed, indicating differences between the processes *in vivo* and *in vitro*. As a consequence, further attention was concentrated on *in vivo* studies, focusing on additional apoptotic processes (involution of the secretory mammary gland or of the ovary removed at different stages of pregnancy or 3–6 days after parturition). In these models, activity of the *TGM2* promoter (as β-galactosidase induction) also increased along induced atrophy. The extension of the analysis to specific tissues taking into account the activity of the TG2 promoter β-galactosidase and of enzymatic endogenous TGase revealed that thymus, spleen, small intestine and kidney at final stages of differentiation (having endogenous apoptosis) expressed high β-galactosidase activity, whereas brain, lungs, heart and liver did not, suggesting a tissue-specific effect. This investigation disclosed that the construct was able to induce *in vivo* cell death in the endocrine-triggered apoptotic system and that the 3.8 kb fragment contained sequences required for apoptosis-specific expression *in vivo*. Among these, the retinoid response element is the only one characterized while sequence comparison displayed species-specific elements of functional differentiation, including one potential glucocorticoid response element and one potential Ap1-binding site. None of these elements supported apoptosis of thymocytes *in vitro*, confirming major differences between the pathways involved *in vitro* and *in vivo*.

The availability of new bioinformatic resources and databases comprising data from ChIP-seq and transcription factor knockout allowed analysis of regulatory genome-wide and expression-based networks. In the UCSC Genome Browser (https://genome.ucsc.edu/), the *TGM2* gene is located on chromosome 20 at chr20:36756864-36793700, identified among the sequences and datasets of Human GRCh37/hg19 Assembly. Its size is 36 837 bp (including UTRs) and the coding exon positions start and stop at chr20:36758621-36793600 for the full-length TG2 transcript. A CpG island (long 318 bp and constituted of 214 CpG) is present near the transcription starting site at chr20:36793550-36793867, and additional features of the 5′ upstream region are specific modifications of histones (chiefly methylation and acylation as histone marks), responsible for regulation of gene expression and for chromatin access. A particular density of the histone

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marks H3K4Me1 and H3K4Me3 (methylation of lysine 4 of histone H3) and H3K27Ac (acetylation of lysine 27 of histone H3) was detectable by sequencing assays in several cell lines. Usually, the marks H3K4Me1 and H3K4Me3, respectively, interest regulatory regions and the promoter, whereas H3K27Ac mark localizes in active regulatory elements. The 5′-flanking sequences of *TGM2* display also DNase I clusters that show hypersensitivity to enzymatic cleavage, a feature of promoter regions, as derived from many cell lines. At the same time, in the 1.53 kb fragment of the promoter, we can see the tracks from transcription factor ChIP-seq (161 Factorbook Motifs from ENCODE assayed), which are represented with grey boxes, where the darkness of the box is proportional to the signal intensity observed at that position. A schematic representation of the promoter is depicted in Figure 4 and the long list of transcription factors in Supplementary Table S2.

Along with these studies on the *TGM2* promoter, investigations identified eRNAs of *TGM2* engaged in gene regulation by recruitment of insulator CTCF in intergenic regions of thymocytes triggered to death by treatment with TGF-β, dbcAMP or retinoids [62]. Three eRNAs functioned as retinoid-selective enhancers located at +30, −7.9 and −28 kb and two additional at −13 and −20 kb responsive to TGF-β, as well as to dbcAMP. The last two regions showed DNase I hypersensitivity, histone acetyltransferase enrichment and binding of P300 and CBP in response to the activators. Combined treatment with 9cisRA, dbcAMP or TGF-β further increased the levels of the eRNAs located at −13 and −20 kb, although the eRNA at −20 kb apparently has a 'shadow' like role in regulation of *TGM2* gene expression, interfering with the intronic and 3′-UTR region. The synergic effects of TGF-β and retinoids appear associated with recruitment of RXR, SMAD4, P300 and CBP, whereas the interaction between adenylate cyclase and retinoid signalling pathways did not associate with enhanced CBP or P300 binding, despite a higher binding of CREB. Along with its role as a CBP co-activator, CREB can interact with several CREB-regulated transcription co-activators and with other transcription factors regulated by the adenylate cyclase signalling pathway. Only CREB1 motif has been found at the enhancer located −20 kb from the *TGM2* gene and the CREB-binding validated by ChIP-qPCR.

Other studies focused on consensus sequences distant from the *TGM2* gene but involved in its regulation. Thus, Ribas et al. [63] checked the oestradiol induction of luciferase plasmids containing 2 kb (with consensus oestrogen response element, ERE) or 1 kb (without ERE) fragments of *TGM2* promoter. Results suggested that the stimulation by oestradiol with the ERE-lacking plasmid was limited and similar conditions appeared to correlate with atherosclerotic lesions in the ERα$^{-/-}$ mice.

Since ChIP-seq experiments (https://genome-euro.ucsc.edu and http://regulome.stanford.edu/snp/) have disclosed the presence of binding sites for transcription factors and the SNPs in the same regions, we have summarized this information in Supplementary Table S2.

Differential start and selection of exons and alternative splicing events generate *TGM2* variants and influence gene expression

We have analyzed the *TGM2* gene for the occurrence of variants by alternative splicing events as it occurs in many other genes because multiple forms of the protein were already detected years ago (reviewed by Lai and Greenberg) [18] and investigated for their catalytic properties [14]. The *TGM2* gene includes 12 introns and a very long 3′-UTR sequence. We have now explored the issues of TG2 variants at the nucleic acid level in the structure of *TGM2* (Figure 5), taking advantage of data reported in the Genome Browser and NCBI web sites. These data indicate that the gene sequence is transcribed to generate multiple mRNAs that include: (i) the variant 1, coding for the canonical TG2 (also named isoform a, isoform 1, tTG and TGC) which is 5144 bp long; (ii) the predicted variant X1 (3998 bp, supported by RNA-seq alignments); (iii) the variant 5 (5127 bp), which is based on similar transcript alignments presenting different 5′-UTR sequences in the first exon, and for the X1 variant, a shorter transcript at the 3′-UTR region; (iv) the variant 2 is stopped at 28 630 nt, after exon X, presenting a poly(A) site and encoding for the isoform b (also named isoform 2, TG-S and TGH) of 548 AAs, similar to TG2 till 538 AA; (v) another polyadenylated transcript encodes TGH2 (also named isoform 3), the shortest form among the TG proteins, containing six exons and identical with the first 286 AAs of canonical TG2, translated from a transcript of 2010 bp, which is stopped at 21 129 nt, causing a stop codon at 349 AAs; (vi) the shortest detected mRNA is produced from the last 5289 bp of the *TGM2* gene, it being constituted of the exons XI, XII and XIII including the 3′-UTR for the most part of its length, as reported in 'NEDO human cDNA sequencing project' (GenBank: AK126508.1); (vii) finally, there are the variants 3 (4901 bp) and 4 (4964 bp) which lack, respectively, exon III and exon II, and were revealed by

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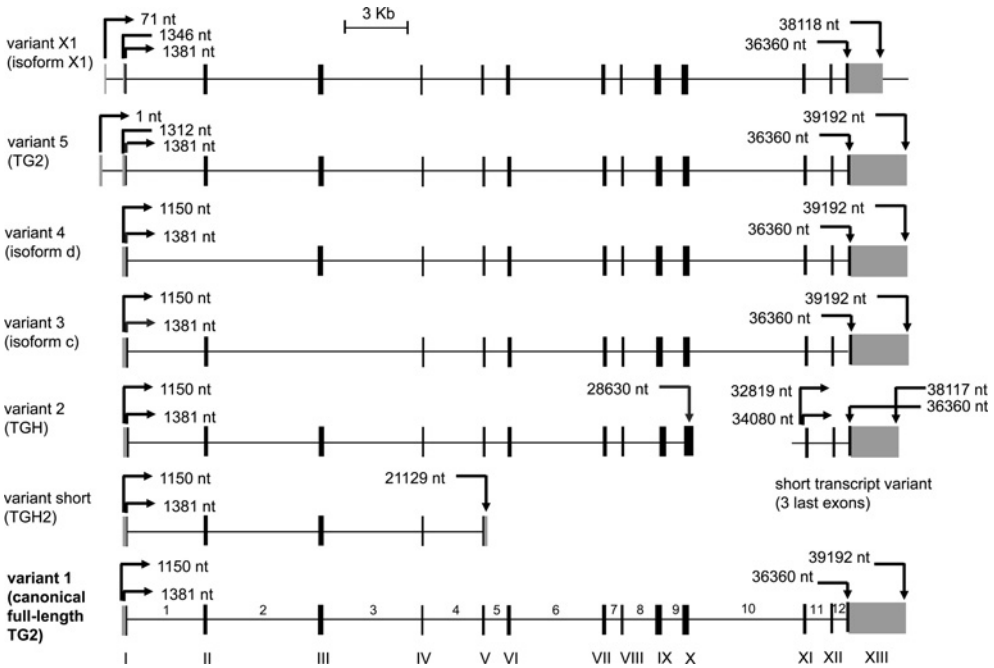

Figure 5. *TGM2* gene and its transcriptional variants.

Grey, mRNA untraslated exon sequences; black, coding sequences; arrows, start and stop of transcription/translation. Roman numerals, exons; arabic numerals, introns. nt, nucleotide. Respective coded isoforms are indicated and their functional features are described in the text.

RNA-sequencing. In this context, it will certainly be necessary to investigate the rules that govern selection of different splicing points.

The first variants identified were described in 1996 by Fraij and Gonzales [64], who demonstrated by Northern blot the presence of three different *TGM2* mRNAs in human erythroleukemia (HEL) cells. Cloning, *in vitro* transcription, translation and immunoprecipitation analyses made it possible to define and compare TG2 and TGH mRNAs with the shorter TGH2 transcript. These molecules derived by alternative splicing of a pre-mRNA and presented alternative polyadenylation sites. Both TGH and TGH2 mRNAs were increased in the HEL cells by retinoic acid (RA) treatment.

Subsequently, additional variants possibly involved in the regulation of microcirculation/inflammation were detected by Lai et al. [65] who discovered two GTP-independent forms of TG2 (*tTGv1* and *tTGv2*, see Figure 1) in leukocytes, endothelial cells and vascular smooth muscle cells (VSMCs). These variants did not form by proteolysis but derived from polyadenylated mRNAs alternatively spliced at sites within exons XII and XIII (Figure 1), so that variants *tTGv1* and *tTGv2* are composed of 674 and 645 AAs, respectively, with 622 AAs identical with the sequence of TG2 and different C-terminal sequences of 52 and 23 AAs, instead of 687 AAs as canonical TG2. They were expressed predominantly in the cytoplasm of HUVEC and in the nucleus of VSMCs achieving levels of *tTGv1* and *tTGv2* that were 10- and 60-fold lower than TG2 in the cytosol of HUVECs and 7- and 45-fold lower in nuclear fractions of VSMCs. In contrast, in mononuclear cells from healthy subjects, *tTGv1* presented at ∼10 fold higher levels than TG2, whereas *tTGv2* was not detected. In addition, *tTGv1* expression was not significantly modified when the promyelocytic leukaemia HL-60 cells were induced by RA (6 μM for 16 h), whereas TG2 protein increased at least 10-fold. These alternative transcripts code *tTGv1* and *tTGv2* isoforms which display lower binding of a fluorescent GTP analogue and hydrolyze GTP at a higher rate than TG2. They showed GTP insensitive transamidase activity, lower stability and half-life than TG2, as if the modified GTP-binding region could affect the degradation pathway [65,66]. These data referring to the altered GTP-binding and TGase activities of the shorter TG2 isoforms contrast with those obtained by Antonyak et al. [67], who affirm that cytotoxic effects of short isoforms to cells are independent of their TGase activity. There are possible reasons for this discrepancy since the authors employed a different

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GTP affinity-labelling assay to evaluate GTP-binding and transamidation activities were determined on the same cell lysates by assaying incorporation of 5-(biotinamido)pentylamine into proteins. The differences further include the presence of a fusion Myc-tag in the TG2 and TGH (358 AAs like TG2 plus 10 AAs) constructs, the use of the mutants S171E and C227V, as well as the choice of 657 AAs isoform lacking the PLCδ1 interaction sequence V665-K672, whereas *tTGv1* and *tTGv2* do not contain tag elements and include at the C-terminus 52 and 23 AAs after the first 622 AAs of TG2 [65].

TG2 variants in neurodegenerative diseases or animal models and loss of sequences at the C-terminal domain

The mechanism of isoforms' generation is largely linked to events of exon selection so that variant proteins can originate by RNA alternative splicing at exons VI and X [12] but also by curious processes of 'exon swapping' which involve reversible use of splicing sequences that code alternatively for long/short variants. These 'swapping' variants are prevalently involved, respectively, in protein transamidation and in G-protein signalling assuming a general regulatory role as suggested for the deposition of aggregated *tau* proteins in Alzheimer's disease (AD) [68]. Brain samples from patients with ante-mortem diagnosis of AD, compared with age-matched non-demented subjects, present selective alterations in TG2 expression which include both TG2 and short TGH isoforms that co-localize with *tau* in neurofibrillary tangle inclusions in hippocampal neurones along with elevated transamidating activity, a hallmark of neuronal degeneration. In this context, the role played by TG2 has been summarized by Min and Chung [69], who directed attention to the substrates of the transamidation activity, including amyloid-β, α-synuclein, mutant huntingtin and to ALS-linked *trans*activation response, in addition to *tau*, that could be modified by TG2 inhibitors.

Analogous changes in TG2 expression with increased activity in neurodegenerative states are also reported in traumatic CNS injury, in HD and in other neuronal diseases with nuclear inclusion bodies derived from elevated TG2 and TG2-catalyzed aggregation of proteins via isopeptide bonds. This opens a new perspective on the involvement of the potential GTP-independent function of the TGH isoform claimed to be involved in molecular mechanisms producing neurotoxic aggregates.

It must also be considered that variants with altered C-terminal regions may display modified interactions with partner proteins as PLCδ1 or GPR56 (a member of the G-protein receptor family) which interacts with the C-terminal β-barrel of TG2 through their N-terminal domain [65]. Conversely, the ability of the TG variants to bind FN is not significantly reduced, as in the case of the protective antiapoptotic properties in HEK293 cells, because these functions are largely ascribed to the N-terminal moiety.

Analogous splicing events occur in rat brain astrocytes treated with the inflammatory cytokines IL-1β or TNF-α, probably as a response to injury [70]. TG2 translocates into the nucleus (probably through involvement of importin-α3 and/or PLCδ1); the tendency of the TG2 variants towards a specific nuclear location suggests that they might play specific function in various intracellular compartments. This might happen in specific kinds of cells (HD neurones, VSMCs and rabbit hepatocytes), in leukaemia cells undergoing RA-induced differentiation or in ethanol- or free fatty acid-treated hepatic cells and in patients affected by liver diseases [71]. From this perspective, it would be interesting to investigate the eventual kinase activity of these TG2 variants and whether they might phosphorylate histones to control chromatin structure, as claimed in the nuclei of breast cancer cells [72], a controversial issue. Indeed, it was suggested that in nuclei, TG2 may phosphorylate p53, decreasing the ability to interact with Mdm2, and Rb, affecting the differential nuclear/cytoplasmic location and destabilization of its complex with E2F1 [73].

Beside modifying nuclear translocation of TG2 with consequences on gene expression activity, the loss of sequences at the C-terminal domains should affect the different GTP-bound conformations of *tTGv1* and *tTGv2* as well as of other short isoforms, and consequently, interactions of TG2 with the no-longer inhibited PLCδ1 [65]. Biological functions of PLCδ1 in cytosol and nucleus should be modified by the incorrect GTP-mediated interaction of TG2 and by the altered GTPase activity. Eventual involvement of TG2 in cell-cycle control and in α1-adrenergic-mediated signalling suggests the relevance to investigate further the functions of TG isoforms. Notably, the concentrations of free Ca^{2+} ($\sim 10^{-7}$ M) and of GTP (~100–150 μM) prevailing in the intracellular compartment are generally considered to prevent TGase activity and cross-linking of intracellular proteins; however, this is unlikely to be the case for the *tTGv1* and *tTGv2* variants which could be activated by transient Ca^{2+} increase and thus play a role in the NF-κB Ca^{2+} activation since they are largely insensitive to inhibition by GTP.

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Alternative splicing transcripts of TG2 in normal and neoplastic conditions

The ability of cells to employ alternative splicing as a means to produce isoforms of proteins in altered physiological or pathological conditions led us to also investigate the roles of variants during neoplastic transformation. Notably, tumours commonly display higher levels of TG2 protein than cognate tissues. The question therefore of the relative contributions of individual variants was explored recently by quantifying TG2 transcripts by RT-qPCR [74] in normal tissues and in related cancer cell lines employing specific primers for the isoforms TG2, TGH, TGH2, *tTGv*1 and *tTGv2*. In addition, studies were also carried out in a 'functional' approach aiming to correlate the differential expression of variants with the phenotypic behaviour of tumours.

In the first approach, studies of TG2 expression in normal tissues demonstrated that full-length TG2 mRNA is expressed at the highest level, followed by TGH, *tTGv1*, *tTGv2* and finally TGH2 with consistent expression of most isoforms in placenta, lung and heart. Also, liver, prostate, trachea, uterus and retina have substantial levels of TG2 variants, with low levels in brain and in testis, where actually *tTGv2* was undetectable. Definitely higher levels of TGH2 mRNA were found in foetal rather than adult liver. In general, there is a linear correlation between expression of canonical TG2 and of variants, with TG2 accounting on average for 84% of the total expression of the gene in normal tissues.

In relation to cancer, the study took into account several cell lines for breast, prostate, melanoma and head and neck squamous cell carcinoma. Most cell lines expressed the full-length transcript, which was particularly high in prostate cell lines DU145 and OPCT1, in breast cell line MDA231 and, in general, in melanoma cell lines, whereas it was lower or undetectable in head and neck cancer cells. In breast cancer cells, full-length TG2 mRNA was present, respectively, at high, intermediate or barely detectable levels in MDA231, MDA468 and T47D cells, and it was also highly expressed in MCF7 cells, despite previous contrasting reports [75,76]. Other variants follow parallel patterns, but for a constantly lower expression of TGH mRNA compared with full-length TG2, and minimal expression of TGH2 mRNA with the exception of the prostate cell lines PC3 and OPCT1. Levels of the transcript variants *tTGv1* and *tTGv2* were more variable with preferential expression of *tTGv1* over *tTGv2* in prostate and the reverse in breast, while they tended to accumulate at high levels in the melanoma cell lines. These results suggest a wide dysregulation of alternative splicing of *TGM2* in cancer, with a general significant correlation between full-length TG2 mRNA and the transcript for TGH. On average, the first contributed to 57% (±33%, SD) of the total gene expression, while no correlation resulted with other isoforms, another indication of variability of *TGM2* splicing among cancer cell lines. *TGM2* expression was also analyzed in prostate clinical cancer samples compared with normal tissue. These data presented a higher level of TGH and TGH2 transcripts than *tTGv1* and *tTGv2* compared with full-length TG2 in prostate cancer, albeit only a few samples were analyzed.

Investigations on the functional association of specific variants with biological behaviours of tumours are expanding. In this context, Minotti et al. [77], using bioinformatic tools, have analyzed the splicing index of the TG2 variants in more than 350 samples of cancer and observed a correlation with altered expression of *TGM2* and its variants in gastric, pancreatic and renal cancers. Additional studies instead correlated expression of *TGM2* with other parameters of cancer growth, such as oxygen sensitivity, tendency to epithelial–mesenchymal transition (EMT) and malignant spreading, and sensitivity to therapy. As a therapeutic approach to correct or reduce splicing events redirecting the expression towards a specific isoform, antisense and complementary splicing–switching oligonucleotide could be used. In the case of *TGM2* gene, molecules to modified gene expression have been addressed only against the full-length mRNA. An example is represented by a study about the expression of full-length TG2 and of four *TGM2* variants in malignant pleural mesothelioma (MPM) by Zonca et al. [78], who observed that the expression of canonical TG2 was increased in epithelioid-derived cells and biphasic MPM cells compared with mesothelial ones (9-fold and 22-fold increase, respectively). The expression of TG2 variants and overall modulation of *TGM2* provided clues to investigate disease progression and effects of suppression of TG2 by means of siRNA on a clone of MPM cells, which display the highest levels of the protein. Silencing TG2 significantly shortened cell survival in hypoxia with only moderate effects in normoxic condition, while increased proliferation was observed in hypoxic cells transfected with TG2 tagged to GFP. Hypoxia modulated these processes as proved by HIF-2α regulation of TG2 mRNA expression following induction of endothelial PAS domain protein 1 and its silencing with siRNA, as confirmed by use of cell-permeable selective TG2 inhibitor. The expression of TG2 and the shift in variants influence MPM cells to hypoxia and their inhibition might represent a strategy to treat tumours undergoing hypoxia during progression, or resistance to radio/chemotherapy.

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The elevated expression of *TGM2* in tumours and the dysregulated occurrence of variants might correlate with the clinical outcome and specific effects such as EMT, abundance of cancer stem cells and sensitivity to therapy and apoptosis. For instance, the long/short TG isoforms can correlate with sensitivity to chemotherapy and resistance to treatment with retinoids improving survival in neuroblastoma-affected patients. Retinoids induced the expression of both TG2 and TGH, but each of them produced different effects on cell cycle. In particular, retinoid induction of TG2 appeared to associate with chemoresistance and not with cell differentiation, whereas increase in TGH expression promoted cell growth arrest also when simultaneously TG2 repression was triggered by specific siRNA in the retinoid-treated cells [60]. Furthermore, the expression of the above variants is controlled by the oncoprotein Myc that contributes also to the sensitivity of the cells to treatment with RA.

In addition, most research concerning the expression of TG2 in cancer focuses on the level of the full-length transcript or protein and on modulation of tumour kinetics. The general aim was to shed light on rational drug administration and related pathways, such as regulation linked to NF-κB and TGF-β. The roles of TG2 in EMT and drug resistance in breast cancer were examined in experiments on cell death induction by combining docetaxel treatment with suppression of *TGM2* using specific siRNA *in vitro* and *in vivo*. In nude mice xenograft models, down-regulation of TG2 promoted apoptosis, inhibited tumour growth and improved the anticancer effect of docetaxel [79]. In the same manner, in ovarian cancer, the levels of TG2 protein and activity correlated with cell survival, adhesion to matrix and invasiveness, as confirmed by knockdown of TG2 in Hey8 cells treated with docetaxel after adhesion to FN substrate [80]. These effects were apparently mediated by TG2 acting as a coreceptor to integrin and correlated with the phosphatidylinositol 3-kinase/Akt pathway. For the same reason, in the SKOV3ip1 cells, up-regulation of TG2 led to adhesion to matrix independently of the transamidation activity, but with increased invasive potential. These effects were reproduced *in vivo* by treating mice with liposomes containing TG2 siRNA, with synergic effect on apoptosis by combination with docetaxel. Other approaches to modulate TG2 gene expression in ovarian cancer cell lines and in transplanted mice were based on suppression by antisense construct (AS-TG2) or stable expression of the full-length transcript [81]. These experiments demonstrated that TG2 expression associated with the EMT phenotype as proved by the biomarker assay with primary tumour size in xenograft mice that correlated with mesenchymal transition and invasiveness [81], with raised and decreased levels, respectively, of E-cadherin and vimentin. In addition, TG2 expression correlated with the E-cadherin transcriptional repressor Zeb1, whose levels were reversed by overexpression of p65 leading to an invasive phenotype.

In xenograft models, the oestrogen-independent growth of MCF7 breast cancer cells overexpressing TG2 supported the concept of the enzyme influence on cell invasiveness and NF-κB pathway [82] that increased following secretion of IL-1β and IL-6 to promote EMT, stem cell-like phenotype and decline in cell-to-cell junction. The involvement of TG2 in these effects in cells stimulated with IL-1β was proved by treatment with TG2 inhibitors. It was demonstrated that IL-1β activated NF-κB signalling in the presence of TG2 by means of luciferase assay and that this was necessary to induce IL-6 expression in MCF7 cells. Thus, TG2 behaved as a positive servomechanism between NF-κB and IL-6/STAT3 to mediate cancer cell aggressiveness and hormone-independent tumour growth. In agreement, the synergistic reciprocal influences between TG2 and NF-κB occur through two NF-κB activation pathways, either TG2-independent (IκK-dependent) or TG2-dependent (IκK-independent). In the TG2-dependent way, the dissociation of IκBα from the NF-κB p65/p50 complex is induced by phosphorylation bringing about nuclear translocation of TG2 associated with p65/p50 to activate constitutively the antiapoptotic Bcl-xL and XIAP genes. TG2 inhibitors ameliorated this response to anticancer drugs [83]. In addition, TG2 may affect NF-κB signalling cross-linking IκBα into complexes (stabilized by isopeptide bonds between K177 and Q267 of IκBα) that contribute to the inflammatory process [84]. Finally, *TGM2* gene is itself a transcriptional target of NF-κB producing a molecular feedforward loop where TG2 induces NF-κB activation, which up-regulates *TGM2* expression [85]. In this context, NF-κB binds two consensus sequences in *TGM2* [56,86], and the methylation status of the gene affects negatively the activation of the TG2/NF-κB signalling loop. There is also evidence that overexpression and activation of NF-κB subunit p65 correlates with tumour grade in glioma with aberrant hypermethylation of *TGM2* in primary brain tumours, whose epigenetic silencing is associated with extended survival. Indeed, TG2 confers resistance to selected drugs commonly used as chemotherapeutic agents, particularly doxorubicin and bifunctional alkylator lomustine without causing a general drug resistance. Moreover, TG2-induced chemoresistance in glioma cells transfected with the inactive W241Q mutant presents similar resistance to doxorubicin to that of cells transfected with wild-type TG2, so that catalytic activity is not required for this effect [53]. Again, analysis of variants was not included in the study.

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Other investigations employing the gene silencing approach concerned the latency of transamidating activity [14] and the possible promotion by cellular concentrations of Ca^{2+} and GTP, which can be eventually switched-on in neoplastic cells following altered Ca^{2+} homeostasis or alternative signalling pathways [87]. This last study, based on stable transfection of MCF10A cells with wild-type or inactive C277S mutant, proved that the catalytic domain was not required for constitutive activation of NF-κB, which therefore does not strictly require processing of IκBα. Down-regulation of TG2 by interference with specific shRNA resulted in decreased nuclear location of p65/RelA and increased IκBα in TG2-expressing cells. In this way, IκBα is induced independently of TG2 activity and of up-regulation of NF-κB target genes, as confirmed by treatment with p65/RelA-specific siRNA or by loss of TG2/IκBα complex in cells transfected with C277S mutant. This event suggests that interactions with TG2 accelerate IκBα degradation by the proteasomal pathway. An association of the HIF-1α pathway in the TG2-induced NF-κB activation is supported by the increase of HIF-1α mRNA in non-tumorigenic MCF10A cells transfected with the TG2-C277S mutant and in revertant MCF7 cells constitutively expressing TG2. The HIF-1α mRNA levels were decreased by TG2-specific shRNA and ChIP assay recognized p65/RelA at the NF-κB-binding site in the gene promoter. Finally, this induced down-regulation of HIF-1α, associated with a decline of Zeb1, Zeb2 and Twist, which therefore act as promoters in chemoresistance and EMT invasive phenotype.

Up-regulation of TG2 and remodelling of ECM during EMT are brought about by TGF-β1, a cytokine that activates cell proliferation and inhibits inflammation, under the control of TG2 that inactivates companion proteins in oxidative, nitric oxide and mechanical stress [54]. TG2 employs its control function by cross-linking of TGF-β1-associated proteins; indeed, there is evidence that TG2 alters TGF-β1 activation in the experimental model of diabetic nephropathy [88], where TG2 inhibitors reduce the level of active TGF-β1 in kidney, a process dependent on NF-κB signalling sustained by a loop in which TGF-β1 and TG2 expression appear reciprocally regulated [3].

In conclusion, there are a lot of fields associated with cancer, neurodegenerative, inflammatory and rare diseases, where the possible function of TG2 transcriptional variants and truncated isoforms need further be explored along with molecular mechanisms leading to their expression.

Effects of regulatory RNAs on post-transcriptional modulation of TGM2:miRNAs, their mRNA target sequences and the relation to SNPs

Within our discussion of the regulation of the *TGM2* gene, we need to mention the roles played by the regulatory RNAs, microRNAs (miRNAs, miRs) and non-coding RNAs. The miRNAs are short nucleotide sequences that specifically interact with mRNA molecules to modify their stability and affect translation. In the *TGM2* transcripts, there are many highly conserved putative target sites of miRNAs. Most of them are located within the 3′-UTR, but not in the introns with the exception of the upstream region flanking the short transcript constituted by the last three exons and the portions downstream of TGH and TGH2 transcripts. Only a few miR target sequences occur in exons. In Table 2, we have listed all families of highly conserved miRs targeting *TGM2* gene reported in the Genome Browser web site. Among these, some have a 'good score' (miR support vector regression, SVR score of less than or equal to −0.1) on microRNA.org web site. We report, in Supplementary Table S3, the miRNAs we have identified as involved in modulation of *TGM2*, along with those that target sites in the 3′-UTR of other members of TGase family, although there are no studies about possible mechanisms of post-transcriptional multi-target modulation.

Despite a large number of putative miRs that could interact with the *TGM2* transcript, only a few studies are reported in the literature dealing with these regulated functions, mostly in relation to cancer. Eom et al. [89] investigated the effects of miR-218 and miR-181a on the signalling by the high-affinity receptor for IgE (FcεRI), where TG2 was induced upon antigen stimulation in a model of mast cells mediating murine allergic inflammation. *In vivo* TG2 can interact with FcεRIβ in the same model promoting progressively growth of primary cutaneous lymphoma and increased metastatic potential of melanoma cells through activation of EGFR and FcεRIβ. Elevated levels of miR-218 reduced TG2 expression and allergic inflammation, whereas their decline restored these effects to control levels. MiR-181a behaved the same way. By means of luciferase vectors containing the 3′-UTR sequence of murin TG2, the authors demonstrated that miR-218 and miR-181a regulate *TGM2* expression. However, target sites recognizing miR-218 and miR-181a are not present in the

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Table 2 miRNAs in 3′-UTR of *TGM2*

All the indicated miRNAs in the columns are reported on the Genome Browser web site (https://genome-euro.ucsc.edu/). Particularly, we list in a further column those also reported as conserved miRNAs with a good score SVR (less than or equal to −0.1) on microRNA.org — Targets and Expression web site (http://www.microrna.org/microrna/home.do). Finally, among these we listed the miRNAs validated by experimental works and reported in the literature. Underlined miRs indicate that miRs validate using luciferase vector containing 3′-UTR of murine *TGM2*, while bolded miRs indicate that miRs validate on human TG2 3′-UTR target.

Part 1 of 2

Gene position	Highly conserved miRNAs reported only from the Genome Browser	Highly conserved miRNAs with a good score from the Genome Browser and microrna.org	Experimental validated miRNAs (miRSVR score)
3′-UTR of TG2	miR-27c/27a-3p; miR-128/128ab; miR-135ab/135a-5p; miR-122/122a/1352; miR-143/1721/4770; miR-34ac/34bc-5p/449abc/449c-5p; miR-129-5p/129ab-5p; miR-23c/23b-3p; miR-503; miR-125a5b-5p/351/670/4319; miR-204/204b/211; miR-129ab-5p; miR-135a-5p; miR-24/24ab/24-3p; miR-143/1721/4770; miR-761/3619-5p	miR-214; miR-377; miR-613; miR-206; miR-1; miR-135ab; miR-28-5p; miR-708; miR-129-5p; miR-23ab; miR-143; miR-135ab; miR-27ab; miR-320abcd; miR-359	**miR-19a** [90] (−0.317) **miR-19b** [90] (−0.317)
The shortest transcript (without the exon sequence)	**5′ upstream:** miR-150/5127; miR-218 [89]/218a; miR-125ab-5p/351/670/4319; miR-503; miR-205/205ab; miR-383; miR-338/338-3p; miR-761/3619-5p; miR-190/190ab; miR-99ab/100; miR-93/93a/105/106a/ 291a-3p/294/295/302abcde/372/ 373/428/519a/520be/520abc-3p/ 1378/1420ac miR-1721/4770; miR-128/128ab; miR-141/200a; miR-142-3p **3′ downstream:** miR-27c/27a-3p; miR-128/128ab; miR-135ab/135a-5p; miR-122/122a/1352; miR-143/1721/4770; miR-34ac/34bc-5p/449abc/449c-5p; miR-129-5p/129ab-5p; miR-23c/23b-3p; miR-503; miR-125ab-5p/351/670/4319; miR-204/204b/211; miR-129ab-5p; miR-135a-5p; miR-24/24ab/24-3p; miR-143/1721/4770; miR-761/3619-5p	**3′ downstream:** miR-214; miR-377 miR-613; miR-206; miR-1; miR-135ab; miR-28-5p; miR-708; miR-129-5p; miR-23ab; miR-143; miR-135ab; miR-27ab; miR-320abcd; **miR-19ab** [90]; miR-359	
Downstream TGH	miR-17-5p/20b-5p/427/518a-3p/519d; miR-93a/105/106b/ 291a-3p/294/295/428/519a/1378/1420ac	miR-223; miR-140-5p; miR-377; miR-340; miR-20ab; miR-17; miR-106ab; miR-93; miR-519d; miR-372; miR-520acd-3p; miR-520be; miR-302abcde; miR-373	
Downstream TGH2	miR-4262; miR-1721/4770; miR-150/5127; miR-449-5p; miR-218a; miR-129ab-5p; miR-122/122a/1352; miR-130ac/301ab/301b/301b-3p/ 454/721/4295/3666; miR-129ab-5p; miR-338; miR-29d; miR-129ab-5p; miR-193/193b/193a-3p; miR-146c; miR-199ab-5p; miR-125ab-5p/351/670/4319; miR-199ab-5p	miR-181a [89]; miR-218 [89]; miR-181bcd; miR-143; miR-337; miR-342-3p; miR-340; miR-129-5p; miR-371-5p; miR-338-3p; miR-29abc; miR-129-5p; miR-129-5p; miR-145; miR-146a; miR-146b-5p	

Continued

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Table 2 miRNAs in 3′-UTR of *TGM2* Part 2 of 2

Gene position	Highly conserved miRNAs reported only from the Genome Browser	Highly conserved miRNAs with a good score from the Genome Browser and microrna.org	Experimental validated miRNAs (miRSVR score)
Exon II	miR-193/193b/193a-3p; miR-17/17-5p/20ab/20b-5p; miR-93/106ab/427/518a-3p/519d	–	
Exon III	miR-138/138ab; miR-133abc	–	
Exon VI	miR-199ab-5p; miR-193/193b/193a-3p; miR-15abc/16/16abc/195/322/424/497/1907	–	
Exon VIII	miR-126-3p	–	
Exon IX	miR-192/215; miR-183	–	
Exon X	miR-375	–	
Exon XI	miR-124/124ab/506; miR-214/761/3619-5p	–	
Exon XII	miR-128/128ab; miR-18ab/4735-3p	–	
Exon XIII	miR-27abc/27a-3p; miR-217/791/3679-5p; miR-214/761/3619-5p	–	

3′-UTR of the human transcript coding for the full-length isoform, but they are present in the 3′-UTR of the transcript coding for the TGH2 isoform; in addition, a sequence target for miR-218 is located at 5′-UTR of the shortest transcript containing the three exons at the end of the gene (see Table 2).

The role of miR-19 was investigated in a model of human colorectal cancer employing two cell lines, SW620 and SW480, that were derived, respectively, from metastatic lymph nodes and from the primary lesion of an individual patient [90]. At variance with reports in other cancer models, these cell lines express low and high TG2 activity, respectively, along with prevalently full-length TG2 and TGH isoforms correlating with invasiveness. Analyzing 3′-UTR of *TGM2* with a panel of four target prediction algorithms, miR-19a/b were identified as potential regulatory miRNAs, but their possible target sequences are present only in 3′-UTR of the full-length TG2 mRNA. Accordingly, luciferase reporter assays were performed to validate the interaction of miR-19a at the 3′-UTR of TG2. Inversely to full-length transcript levels, miR-19 was up-regulated in SW620 compared with SW480 cells (2.6-fold, miR-19a and 3-fold, miR-19b), in metastatic vs. primary tumours, probably through fragment amplification of chromosome 13, where miR-19a/b are located. In conclusion, an increase of miR-19a can produce the same effects than a TG2 reduction obtained by specific siRNA; in both cases, IL-8 secretion was affected, but conversely could be up-regulated when TG2 levels were restored by TG2-plasmid transfection. The authors underscored that transcription of truncated TG2 isoforms cannot be repressed by miR-19 [90]. Another miR, miR-1285, which is described as an oncosuppressor miRNA, has been implicated in the regulation of *TGM2* in renal cell carcinoma inducing a decline in TG2 expression that is definitely more expressed in cancer than in the normal cells [91] and a concomitant inhibition in cell growth and invasiveness. Target sequence for miR-1285 was at 3′-UTR of TG2 mRNA, as proved by the usual 3′-UTR-TG2-luciferase assay. In addition, a reduction of TG2 mRNA was observed after transfection with miR-1285. The effects on cell proliferation, migration and invasion were assayed demonstrating their relation to support cancer development. MiR-1285 has a good SVR score, but it is not included among conserved target sites of miRs on microRNA.org — Targets and Expression web site. In fact, there is a considerable interest in miR-1285 because it presents four target sites in the 3′-UTR of the full-length of TG2 and two in 3′-UTR of the mRNA coding for TGH2 isoform. In Figure 6, we describe predicted miRs with a good score and highly conserved target sites in 3′-UTR of the full-length mRNA coding for TG2 pointing out those validated by the *in vitro* assay with luciferase under their 3′-UTR control.

Taking into account the combined effects of SNPs and of miRNAs on *TGM2*, it appears that the only SNP that can affect putative target miR sequences on the gene is rs45440600 located inside the miR-218/218a target site and borderline to miR-1ab/206/613 sites, which all bind close to each other. The sequence rs45440600 is in the 5′-UTR region of the short transcript including the last three exons and the 3′-UTR. Notably, miR-1 [92–97], miR-206 [98–102] and miR-603 [103–106] could be important in several disorders including cancer, cardiovascular and metabolic diseases, and apoptosis.

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In addition, the isoforms affected by exon skipping, such as those missing exon II or III, could escape post-transcriptional modulation by predicted miR-193/193b/193a-3p, miR-17/17-5p/20ab/20b-5p, miR-93/106ab/427/518a-3p/519 recognizing target sites in the exon II, and miR-138/138ab, miR-133abc in the exon III. Other transcripts, *tTGv1* and *tTGv2*, skipping the exons XIII and partially XII lost eventually post-transcriptional modulation of the miR-27abc/27a-3p, miR-217/791/3679-5p, miR-214/761/3619-5p (exon XIII), and miR-218a, miR-18ab/4735-3p (exon XII), and others because of their different 3′-UTR region, as reported in Table 2 and Figure 6 (shown by area in yellow).

About the roles of RNA in the regulation of *TGM2*, we want mention lncRNAs: one of them, the lncRNA named LOC107987281 reported on the NCBI database (https://www.ncbi.nlm.nih.gov), is included in the first intron of *TGM2*. It corresponds to an uncharacterized transcript (XR_001754586) 1000 bp long and formed by two exons that fuse to generate the linear lncRNA predicted by automated computational analysis. This sequence derived from RNA-sequencing project carried on different normal tissue samples from human individuals [107]. This lncRNA is correlated with TG2 expression and is associated with specific alternative splicing variants in some type of cancer [77]. Whether or not this RNA molecule plays a role in regulating *TGM2* gene is a matter of recent investigations.

Conclusions and perspectives

The involvement of TG2 in human pathology has long been appreciated as in the case of neurodegenerative disorders (including Alzheimer's, Parkinson and Huntington disease), as has its role as autoantigen for antibodies developed in gluten sensitivity, and in inflammation and tumour biology, including drug resistance, promotion of metastases and EMT. This enzyme has been reported to participate in the pathology for 329 reported diseases (https://www.targetvalidation.org/target/ENSG00000198959/associations), including cardiovascular, epidermal, ocular, renal, metabolic, haematological diseases or pathologies involving the skeletal, nervous, digestive, endocrine, respiratory and immune system. It is important to bear in mind that many pathways linked to TG2 may depend on interaction/intersection with other proteins, e.g. CREB pathway and phospholipase C pathways, Ca^{2+}, cAMP and lipid signalling, regulation by $\alpha 1^{B}$-adrenergic receptors and also respiratory chain electron transport, ATP synthesis by chemiosmotic coupling and heat production. It is highly possible

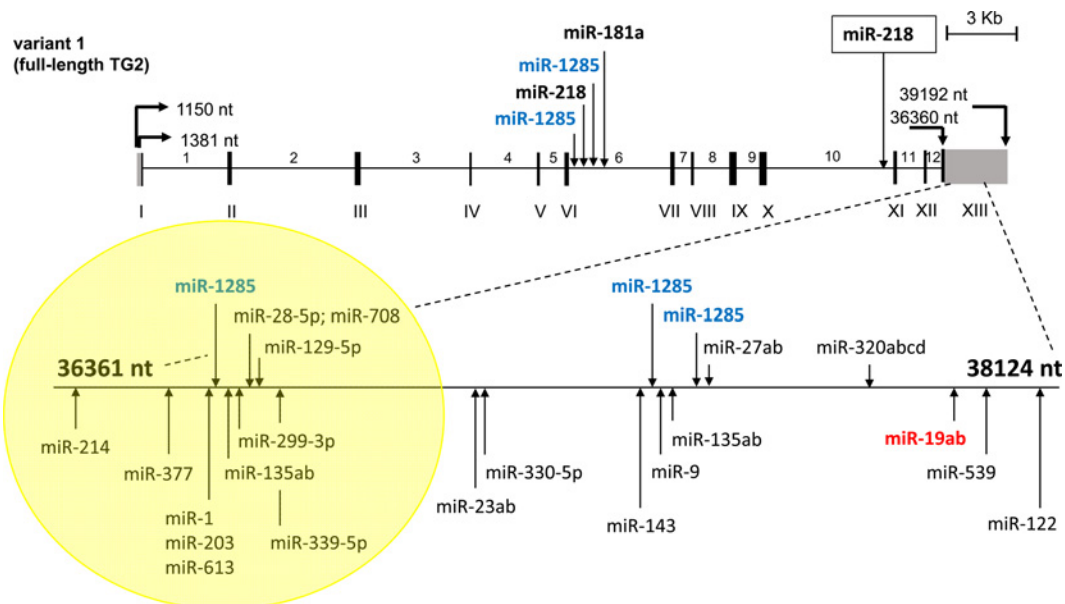

Figure 6. MicroRNA target sites in the 3′-UTR of *TGM2* gene.

Highly conserved miRs with a good score SVR are indicated in the 3′-UTR of *TGM2*, where the yellow circle highlights those lost by alternative spliced *tTGv1* and *tTGv2* variants. In bold, the target sites of validated miRs: blue, miR-1285, which has a good score, but it is no conserved; red, miR-19ab-binding 3′-UTR on human TG2 and black, 218/181a demonstrates to recognizing murine *TGM2*. We have boxed the target site of the miR-218 susceptible to rs45440600 SNP.

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that regulation and modulation of *TGM2* expression could affect heavily these pathways, thanks to its relationships with the signalling of NF-κB, RA, IL-6, TGF-β, HRE, Ap1 and GRE. In this context, it would be relevant to investigate effects of SNPs at transcription factor-binding sites to improve molecular knowledge of DNA/protein interactions, even more so after the studies carried out on SNPs in exonic sequences which bring about no relevant modifications of enzyme functions [18,37,44]. Actually, very few SNPs are associated with pathological phenotypes [43,45,50,51]. Another aspect that should be analyzed is the relationships between transcriptional variants or their ratio of expression levels and functional/pathological conditions. Numerous studies concern alternative splicing events generating *TGM2* transcriptional variants and TG2 isoforms [12,60,65,68,74], which lack functional domains. There is evidence that altered conformations induce significant changes in particular in GTP binding and consequently in the dependence of TGase activity on Ca^{2+} concentration [73]. These aspects are frequently investigated by means of inhibitors against TG2-specific sites [108]. Indeed, the full-length isoform catalyzes Ca^{2+}−dependent post-translational modification of proteins and GTP hydrolysis appointing TG2 as a signalling molecule, while the wild-type enzyme and its truncated/altered isoforms display different effects on biological functions. For instance, full-length TG2 affects PI3-kinase signalling, inducing high levels of Akt, mTOR, and p70 S6-kinase phosphorylation, binding c-SRC and promoting cell survival [109]. In contrast, truncated/altered TG2 isoforms interfere with PLCδ1 with an increase in the rates of apoptosis, autophagy and cell death [110]. An example of 'switch' from expression of full-length TG2 (GTP-dependent) to a shorter isoform of TG2 (GTP-independent) was observed within the first few hours after injury of spinal cord motor neurones in adult rat before cell apoptosis [111]. Despite the large number of reports supporting a role of TG2 in cell death and apoptosis, experiments on transgenic mice models did not fit completely with these evidences since recombinant KO mice lacking TG2 activity because of partial deletion of exons V, intron 5, exon VI, which contains the active site, display normal phenotype as in De Laurenzi and Melino [112]. The animals showed limited residual enzymatic activity in liver and thymus extracts, probably due to contributions by other TGases. Analogous results were reported independently by Nanda et al. [113], who deleted further the active site region extending to include up to exon VIII. Also, in this instance, authors reported no major phenotypic change (notably in adrenergic response and apoptosis in thymus), decreasing — but not suppressing — TG activity in liver and heart, with a virtually complete inactivation of transamidase in fibroblast lysates. On the other hand, it is known that there are compensation mechanisms among different TGase members in skin and autoimmune disease [114].

Among the available strategies to modulate gene expression and to investigate aspects associated with the 'double face' of TG2 [6,11], RNA interference molecules, such miRNAs and siRNA, might be useful tools. The activity of some miRNAs was found to modulate *TGM2* [89–91] possibly with alternations in the ratio of expressed variants; however, looking at 3′-UTR of this gene, many miRNA-binding sites emerged which have not yet been checked for inhibitory action. In spite of all work already carried out, it is evident that much more is required to understand functional details of the *TGM2* gene. In this context, eRNA molecules could represent an additional resource usable to alternate *TGM2* expression and to clarify structural mechanisms and molecular interactions, since they appear to increase upon RA induction [55]. An improved knowledge of the gene regulation will influence the development of new therapeutic approaches and possibly drive the emergence of TG2-related diagnostic markers [13]. It is likely that this intriguing gene will be a model to investigate more generally the mechanisms regulating gene expression; now that, with increasing clarity, it appears to be the target of a wide array of molecules, including eRNA, lncRNA and microRNA, and is the object of alternative splicing events generating transcriptional variants, involved in a high number of pathways, biological processes and diseases.

Abbreviations

AD, Alzheimer's disease; ECM, extracellular matrix; EMT, epithelial to mesenchymal transition; EPAS1, endothelial PAS domain protein 1; ERE, oestrogen response element; eRNAs, enhancer RNA molecules; FcεRI, high-affinity receptor for IgE; FN, fibronectin; HD, Huntington's disease; HDAC1, histone deacetylase 1 protein; HEL, human erythroleukemia cells; HRE, hypoxia-response element; IκBα, inhibitor of nuclear factor-κBα; IL-6, interleukin 6; lncRNA, long non-coding RNA; miRNAs, miRs, microRNAs; MODY, Maturity-Onset Diabetes of Young; MPM, malignant pleural mesothelioma; NES, nuclear export signal; NLS, nuclear location signals; nsSNVs, non-synonymous single-nucleotide variants; PLCδ1, phospholipase Cδ1; PolyPhen, polymorphism phenotyping; RA, retinoic acid; Rb, retinoblastoma protein; SDC4, syndecan-4; SIFT, Sorting Intolerant From Tolerant; SNP, single-nucleotide polymorphism; SVR, support vector regression; TG2,

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type 2 transglutaminase; *TGM2*, transglutaminase 2 gene; *tTGv1*, tissue transglutaminase alternative splicing variant 1; *tTGv2*, tissue transglutaminase alternative splicing variant 2; VSMCs, vascular smooth muscle cells.

Author Contribution

C.M.B. and N.B. drafted the manuscript. S.B. contributed to improve the manuscript.

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Competing Interests

The Authors declare that there are no competing interests associated with the manuscript.

References

1 Beninati, S., Bergamini, C.M. and Piacentini, M. (2009) An overview of the first 50 years of transglutaminase research. *Amino Acids* **36**, 591–598 https://doi.org/10.1007/s00726-008-0211-x
2 Bergamini, C.M., Collighan, R.J., Wang, Z. and Griffin, M. (2011) Structure and regulation of type 2 transglutaminase in relation to its physiological functions and pathological roles. *Adv. Enzymol. Relat. Areas Mol. Biol.* **78**, 1–46 PMID:22220471
3 Eckert, R.L., Kaartinen, M.T., Nurminskaya, M., Belkin, A.M., Colak, G., Johnson, G.V.W. et al. (2014) Transglutaminase regulation of cell function. *Physiol. Rev.* **94**, 383–417 https://doi.org/10.1152/physrev.00019.2013
4 Lai, T.-S., Lin, C.-J. and Greenberg, C.S. (2017) Role of tissue transglutaminase-2 (TG2)-mediated aminylation in biological processes. *Amino Acids* **49**, 501–515 https://doi.org/10.1007/s00726-016-2270-8
5 Tatsukawa, H., Furutani, Y., Hitomi, K. and Kojima, S. (2016) Transglutaminase 2 has opposing roles in the regulation of cellular functions as well as cell growth and death. *Cell Death Dis.* **7**, e2244 https://doi.org/10.1038/cddis.2016.150
6 Beninati, S., Piacentini, M. and Bergamini, C.M. (2017) Transglutaminase 2, a double face enzyme. *Amino Acids* **49**, 415–423 https://doi.org/10.1007/s00726-017-2394-5
7 Pinkas, D.M., Strop, P., Brunger, A.T. and Khosla, C. (2007) Transglutaminase 2 undergoes a large conformational change upon activation. *PLoS Biol.* **5**, e327 https://doi.org/10.1371/journal.pbio.0050327
8 Begg, G.E., Carrington, L., Stokes, P.H., Matthews, J.M., Wouters, M.A., Husain, A. et al. (2006) Mechanism of allosteric regulation of transglutaminase 2 by GTP. *Proc. Natl Acad. Sci. U.S.A.* **103**, 19683–19688 https://doi.org/10.1073/pnas.0609283103
9 Zhang, J., Lesort, M., Guttmann, R.P. and Johnson, G.V.W. (1998) Modulation of the *in situ* activity of tissue transglutaminase by calcium and GTP. *J. Biol. Chem.* **273**, 2288–2295 https://doi.org/10.1074/jbc.273.4.2288
10 Pavlyukov, M.S., Antipova, N.V., Balashova, M.V. and Shakhparonov, M.I. (2012) Detection of transglutaminase 2 conformational changes in living cell. *Biochem. Biophys. Res. Commun.* **421**, 773–779 https://doi.org/10.1016/j.bbrc.2012.04.082
11 Gundemir, S., Colak, G., Tucholski, J. and Johnson, G.V.W. (2012) Transglutaminase 2: a molecular Swiss army knife. *Biochim. Biophys. Acta, Mol. Cell Res.* **1823**, 406–419 https://doi.org/10.1016/j.bbamcr.2011.09.012
12 Lai, T.S. and Greenberg, C.S. (2013) TGM2 and implications for human disease: role of alternative splicing. *Front. Biosci.* **18**, 504–519 https://doi.org/10.2741/4117
13 Agnihotri, N. and Mehta, K. (2017) Transglutaminase-2: evolution from pedestrian protein to a promising therapeutic target. *Amino Acids* **49**, 425–439 https://doi.org/10.1007/s00726-016-2320-2
14 Király, R., Demény, M. and Fésüs, L. (2011) Protein transamidation by transglutaminase 2 in cells: a disputed Ca^{2+}-dependent action of a multifunctional protein. *FEBS J.* **278**, 4717–4739 https://doi.org/10.1111/j.1742-4658.2011.08345.x
15 Belkin, A.M. (2011) Extracellular TG2: emerging functions and regulation. *FEBS J.* **278**, 4704–4716 https://doi.org/10.1111/j.1742-4658.2011.08346.x
16 Fésüs, L. and Piacentini, M. (2002) Transglutaminase 2: an enigmatic enzyme with diverse functions. *Trends Biochem. Sci.* **27**, 534–539 https://doi.org/10.1016/S0968-0004(02)02182-5
17 Wang, Z. and Griffin, M. (2012) TG2, a novel extracellular protein with multiple functions. *Amino Acids* **42**, 939–949 https://doi.org/10.1007/s00726-011-1008-x
18 Király, R., Barta, E. and Fésüs, L. (2013) Polymorphism of transglutaminase 2: unusually low frequency of genomic variants with deficient functions. *Amino Acids* **44**, 215–225 https://doi.org/10.1007/s00726-011-1194-6
19 Milakovic, T., Tucholski, J., McCoy, E. and Johnson, G.V.W. (2004) Intracellular localization and activity state of tissue transglutaminase differentially impacts cell death. *J. Biol. Chem.* **279**, 8715–8722 https://doi.org/10.1074/jbc.M308479200
20 Stamnaes, J., Pinkas, D.M., Fleckenstein, B., Khosla, C. and Sollid, L.M. (2010) Redox regulation of transglutaminase 2 activity. *J. Biol. Chem.* **285**, 25402–25409 https://doi.org/10.1074/jbc.M109.097162
21 Monteagudo, A., Ji, C., Akbar, A., Keillor, J.W. and Johnson, G.V.W. (2017) Inhibition or ablation of transglutaminase 2 impairs astrocyte migration. *Biochem. Biophys. Res. Commun.* **482**, 942–947 https://doi.org/10.1016/j.bbrc.2016.11.137
22 Stamnaes, J., Cardoso, I., Iversen, R. and Sollid, L.M. (2016) Transglutaminase 2 strongly binds to an extracellular matrix component other than fibronectin via its second C-terminal beta-barrel domain. *FEBS J.* **283**, 3994–4010 https://doi.org/10.1111/febs.13907
23 Al-Jallad, H.F., Nakano, Y., Chen, J.L.Y., McMillan, E., Lefebvre, C. and Kaartinen, M.T. (2006) Transglutaminase activity regulates osteoblast differentiation and matrix mineralization in MC3T3-E1 osteoblast cultures. *Matrix Biol.* **25**, 135–148 https://doi.org/10.1016/j.matbio.2005.11.001
24 Auld, G.C., Ritchie, H., Robbie, L.A. and Booth, N.A. (2001) Thrombin upregulates tissue transglutaminase in endothelial cells: a potential role for tissue transglutaminase in stability of atherosclerotic plaque. *Arterioscler. Thromb. Vasc. Biol.* **21**, 1689–1694 https://doi.org/10.1161/hq1001.097063
25 Furini, G., Schroeder, N., Huang, L., Boocock, D., Scarpellini, A., Coveney, C. et al. (2018) Proteomic profiling reveals the transglutaminase-2 externalization pathway in kidneys after unilateral ureteric obstruction. *J. Am. Soc. Nephrol.* **29**, 880–905 https://doi.org/10.1681/ASN.2017050479

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26 Chou, C.-Y., Streets, A.J., Watson, P.F., Huang, L., Verderio, E.A.M. and Johnson, T.S. (2011) A crucial sequence for transglutaminase type 2 extracellular trafficking in renal tubular epithelial cells lies in its N-terminal βa-sandwich domain. *J. Biol. Chem.* **286**, 27825–27835 https://doi.org/10.1074/jbc.M111.226340

27 Siegel, M., Strnad, P., Watts, R.E., Choi, K., Jabri, B., Omary, M.B. et al. (2008) Extracellular transglutaminase 2 is catalytically inactive, but is transiently activated upon tissue injury. *PLoS ONE* **3**, e1861 https://doi.org/10.1371/journal.pone.0001861

28 Jin, X., Stamnaes, J., Klöck, C., DiRaimondo, T.R., Sollid, L.M. and Khosla, C. (2011) Activation of extracellular transglutaminase 2 by thioredoxin. *J. Biol. Chem.* **286**, 37866–37873 https://doi.org/10.1074/jbc.M111.287490

29 Lai, T.-S., Lin, C.-J., Wu, Y.-T. and Wu, C.-J. (2017) Tissue transglutaminase TG2 and mitochondrial function and dysfunction. *Front. Biosci.* **22**, 1114–1137 https://doi.org/10.2741/4536

30 Mastroberardino, P.G., Farrace, M.G., Viti, I., Pavone, F., Fimia, G.M., Melino, G. et al. (2006) 'Tissue' transglutaminase contributes to the formation of disulphide bridges in proteins of mitochondrial respiratory complexes. *Biochim. Biophys. Acta, Bioenergetics* **1757**, 1357–1365 https://doi.org/10.1016/j.bbabio.2006.07.007

31 Peng, X., Zhang, Y., Zhang, H., Graner, S., Williams, J.F., Levitt, M.L. et al. (1999) Interaction of tissue transglutaminase with nuclear transport protein importin-α3. *FEBS Lett.* **446**, 35–39 https://doi.org/10.1016/S0014-5793(99)00018-6

32 Shrestha, R., Tatsukawa, H., Shrestha, R., Ishibashi, N., Matsuura, T., Kagechika, H. et al. (2015) Molecular mechanism by which acyclic retinoid induces nuclear localization of transglutaminase 2 in human hepatocellular carcinoma cells. *Cell Death Dis.* **6**, e2002 https://doi.org/10.1038/cddis.2015.339

33 Mishra, S., Melino, G. and Murphy, L.J. (2007) Transglutaminase 2 kinase activity facilitates protein kinase A-induced phosphorylation of retinoblastoma protein. *J. Biol. Chem.* **282**, 18108–18115 https://doi.org/10.1074/jbc.M607413200

34 Gentile, V., Saydak, M., Chiocca, E.A., Akande, O., Birckbichler, P.J., Lee, K.N. et al. (1991) Isolation and characterization of cDNA clones to mouse macrophage and human endothelial cell tissue transglutaminases. *J. Biol. Chem.* **266**, 478–483 PMID:1670766

35 Fraij, B.M. and Gonzales, R.A. (1997) Organization and structure of the human tissue transglutaminase gene. *Biochim. Biophys. Acta, Gene Struct. Exp.* **1354**, 65–71 https://doi.org/10.1016/S0167-4781(97)00132-2

36 Lu, S., Saydak, M., Gentile, V., Stein, J.P. and Davies, P.J.A. (1995) Isolation and characterization of the human tissue transglutaminase gene promoter. *J. Biol. Chem.* **270**, 9748–9756 https://doi.org/10.1074/jbc.270.17.9748

37 Thangaraju, K., Király, R., Mótyán, J.A., Ambrus, V.A., Fuxreiter, M. and Fésüs, L. (2017) Computational analyses of the effect of novel amino acid clusters of human transglutaminase 2 on its structure and function. *Amino Acids* **49**, 605–614 https://doi.org/10.1007/s00726-016-2330-0

38 Simon-Vecsei, Z., Király, R., Bagossi, P., Tóth, B., Dahlbom, I., Caja, S. et al. (2012) A single conformational transglutaminase 2 epitope contributed by three domains is critical for celiac antibody binding and effects. *Proc. Natl Acad. Sci. U.S.A.* **109**, 431–436 https://doi.org/10.1073/pnas.1107811108

39 Iversen, R., Mysling, S., Hnida, K., Jørgensen, T.J.D. and Sollid, L.M. (2014) Activity-regulating structural changes and autoantibody epitopes in transglutaminase 2 assessed by hydrogen/deuterium exchange. *Proc. Natl Acad. Sci. U.S.A.* **111**, 17146–17151 https://doi.org/10.1073/pnas.1407457111

40 Bian, Y., Song, C., Cheng, K., Dong, M., Wang, F., Huang, J. et al. (2014) An enzyme assisted RP-RPLC approach for in-depth analysis of human liver phosphoproteome. *J. Proteomics* **96**, 253–262 https://doi.org/10.1016/j.jprot.2013.11.014

41 Rodolfo, C., Mormone, E., Matarrese, P., Ciccosanti, F., Farrace, M.G., Garofano, E. et al. (2004) Tissue transglutaminase is a multifunctional BH3-only protein. *J. Biol. Chem.* **279**, 54783–54792 https://doi.org/10.1074/jbc.M410938200

42 Sjöblom, T., Jones, S., Wood, L.D., Parsons, D.W., Lin, J., Barber, T.D. et al. (2006) The consensus coding sequences of human breast and colorectal cancers. *Science* **314**, 268–274 https://doi.org/10.1126/science.1133427

43 Porzio, O., Massa, O., Cunsolo, V., Colombo, C., Malaponti, M., Bertuzzi, F. et al. (2007) Missense mutations in the *TGM2* gene encoding transglutaminase 2 are found in patients with early-onset type 2 diabetes. Mutation in brief no. 982. Online. *Hum. Mutat.* **28**, 1150–1159 https://doi.org/10.1002/humu.9511

44 Thangaraju, K., Király, R., Demény, M.A., András Mótyán, J., Fuxreiter, M. and Fésüs, L. (2017) Genomic variants reveal differential evolutionary constraints on human transglutaminases and point towards unrecognized significance of transglutaminase 2. *PLoS ONE* **12**, e0172189 https://doi.org/10.1371/journal.pone.0172189

45 Bernassola, F., Federici, M., Corazzari, M., Terrinoni, A., Hribal, M.L., De Laurenzi, V. et al. (2002) Role of transglutaminase 2 in glucose tolerance: knockout mice studies and a putative mutation in a MODY patient. *FASEB J.* **16**, 1371–1378 https://doi.org/10.1096/fj.01-0689com

46 Sener, A., Dunlop, M.E., Gomis, R., Mathias, P.C.F., Malaisse-Lagae, F. and Malaisse, W.J. (1985) Role of transglutaminase in insulin release. Study with glycine and sarcosine methylesters. *Endocrinology* **117**, 237–242 https://doi.org/10.1210/endo-117-1-237

47 Iismaa, S.E., Aplin, M., Holman, S., Yiu, T.W., Jackson, K., Burchfield, J.G. et al. (2013) Glucose homeostasis in mice is transglutaminase 2 independent. *PLoS ONE* **8**, e63346 https://doi.org/10.1371/journal.pone.0063346

48 Szondy, Z., Sarang, Z., Molnar, P., Nemeth, T., Piacentini, M., Mastroberardino, P.G. et al. (2003) Transglutaminase $2^{-/-}$ mice reveal a phagocytosis-associated crosstalk between macrophages and apoptotic cells. *Proc. Natl Acad. Sci. U.S.A.* **100**, 7812–7817 https://doi.org/10.1073/pnas.0832466100

49 Bradford, M., Law, M.H., Stewart, A.D., Shaw, D.J., Megson, I.L. and Wei, J. (2009) The TGM2 gene is associated with schizophrenia in a British population. *Am. J. Med. Genet. Part B* **150B**, 335–340 https://doi.org/10.1002/ajmg.b.30813

50 Bradford, M., Law, M.H., Megson, I.L. and Wei, J. (2011) The functional significance of the TGM2 gene in schizophrenia: a correlation of SNPs and circulating IL-2 levels. *J. Neuroimmunol.* **232**, 5–7 https://doi.org/10.1016/j.jneuroim.2010.09.007

51 Tokuriki, N. and Tawfik, D.S. (2009) Chaperonin overexpression promotes genetic variation and enzyme evolution. *Nature* **459**, 668–673 https://doi.org/10.1038/nature08009

52 Lu, S. and Davies, P.J.A. (1997) Regulation of the expression of the tissue transglutaminase gene by DNA methylation. *Proc. Natl Acad. Sci. U.S.A.* **94**, 4692–4697 https://doi.org/10.1073/pnas.94.9.4692

53 Dyer, L.M., Schooler, K.P., Ai, L., Klop, C., Qiu, J., Robertson, K.D. et al. (2011) The transglutaminase 2 gene is aberrantly hypermethylated in glioma. *J. Neurooncol.* **101**, 429–440 https://doi.org/10.1007/s11060-010-0277-7

54 Ientile, R., Caccamo, D. and Griffin, M. (2007) Tissue transglutaminase and the stress response. *Amino Acids* **33**, 385–394 https://doi.org/10.1007/s00726-007-0517-0

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55 Daniel, B., Nagy, G., Hah, N., Horvath, A., Czimmerer, Z., Poliska, S. et al. (2014) The active enhancer network operated by liganded RXR supports angiogenic activity in macrophages. *Genes Dev.* **28**, 1562–1577 https://doi.org/10.1101/gad.242685.114

56 Mirza, A., Liu, S.L., Frizell, E., Zhu, J., Maddukuri, S., Martinez, J. et al. (1997) A role for tissue transglutaminase in hepatic injury and fibrogenesis, and its regulation by NF-kappaB. *Am. J. Physiol.* **272**(2 Pt 1), G281–G288 https://doi.org/10.1152/ajpgi.1997.272.2.G281

57 Lee, J., Kim, Y.-S., Choi, D.-H., Bang, M.S., Han, T.R., Joh, T.H. et al. (2004) Transglutaminase 2 induces nuclear factor-κB activation via a novel pathway in BV-2 microglia. *J. Biol. Chem.* **279**, 53725–53735 https://doi.org/10.1074/jbc.M407627200

58 Rébé, C., Raveneau, M., Chevriaux, A., Lakomy, D., Sberna, A.-L., Costa, A. et al. (2009) Induction of transglutaminase 2 by a liver X receptor/retinoic acid receptor α pathway increases the clearance of apoptotic cells by human macrophages. *Circ. Res.* **105**, 393–401 https://doi.org/10.1161/CIRCRESAHA.109.201855

59 Liu, T., Tee, A.E.L., Porro, A., Smith, S.A., Dwarte, T., Liu, P.Y. et al. (2007) Activation of tissue transglutaminase transcription by histone deacetylase inhibition as a therapeutic approach for Myc oncogenesis. *Proc. Natl Acad. Sci. U.S.A.* **104**, 18682–18687 https://doi.org/10.1073/pnas.0705524104

60 Tee, A.E.L., Marshall, G.M., Liu, P.Y., Xu, N., Haber, M., Norris, M.D. et al. (2010) Opposing effects of two tissue transglutaminase protein isoforms in neuroblastoma cell differentiation. *J. Biol. Chem.* **285**, 3561–3567 https://doi.org/10.1074/jbc.M109.053041

61 Szegezdi, E., Szondy, Z., Nagy, L., Nemes, Z., Friis, R.R., Davies, P.J.A. et al. (2000) Apoptosis-linked in vivo regulation of the tissue transglutaminase gene promoter. *Cell Death Differ.* **7**, 1225–1233 https://doi.org/10.1038/sj.cdd.4400751

62 Sándor, K., Daniel, B., Kiss, B., Kovács, F. and Szondy, Z. (2016) Transcriptional control of transglutaminase 2 expression in mouse apoptotic thymocytes. *Biochim. Biophys. Acta, Gene Regul. Mech.* **1859**, 964–974 https://doi.org/10.1016/j.bbagrm.2016.05.011

63 Ribas, V., Drew, B.G., Le, J.A., Soleymani, T., Daraei, P., Sitz, D. et al. (2011) Myeloid-specific estrogen receptor α deficiency impairs metabolic homeostasis and accelerates atherosclerotic lesion development. *Proc. Natl Acad. Sci. U.S.A.* **108**, 16457–16462 https://doi.org/10.1073/pnas.1104533108

64 Fraij, B.M. and Gonzales, R.A. (1996) A third human tissue transglutaminase homologue as a result of alternative gene transcripts. *Biochim. Biophys. Acta, Gene Struct. Exp.* **1306**, 63–74 https://doi.org/10.1016/0167-4781(95)00219-7

65 Lai, T.-S., Liu, Y., Li, W. and Greenberg, C.S. (2007) Identification of two GTP-independent alternatively spliced forms of tissue transglutaminase in human leukocytes, vascular smooth muscle, and endothelial cells. *FASEB J.* **21**, 4131–4143 https://doi.org/10.1096/fj.06-7598com

66 Bergamini, C.M. (2007) Effects of ligands on the stability of tissue transglutaminase: studies in vitro suggest possible modulation by ligands of protein turn-over *in vivo*. *Amino Acids* **33**, 415–421 https://doi.org/10.1007/s00726-006-0457-0

67 Antonyak, M.A., Jansen, J.M., Miller, A.M., Ly, T.K., Endo, M. and Cerione, R.A. (2006) Two isoforms of tissue transglutaminase mediate opposing cellular fates. *Proc. Natl Acad. Sci. U.S.A.* **103**, 18609–18614 https://doi.org/10.1073/pnas.0604844103

68 Citron, B.A., SantaCruz, K.S., Davies, P.J.A. and Festoff, B.W. (2001) Intron-exon swapping of transglutaminase mRNA and neuronal Tau aggregation in Alzheimer's disease. *J. Biol. Chem.* **276**, 3295–3301 https://doi.org/10.1074/jbc.M004776200

69 Min, B. and Chung, K.C. (2018) New insight into transglutaminase 2 and link to neurodegenerative diseases. *BMB Rep.* **51**, 5–13 https://doi.org/10.5483/BMBRep.2018.51.1.227

70 Monsonego, A., Shani, Y., Friedmann, I., Paas, Y., Eizenberg, O. and Schwartz, M. (1997) Expression of GTP-dependent and GTP-independent tissue-type transglutaminase in cytokine-treated rat brain astrocytes. *J. Biol. Chem.* **272**, 3724–3732 https://doi.org/10.1074/jbc.272.6.3724

71 Kojima, S., Kuo, T.-F. and Tatsukawa, H. (2012) Regulation of transglutaminase-mediated hepatic cell death in alcoholic steatohepatitis and non-alcoholic steatohepatitis. *J. Gastroenterol. Hepatol.* **27**, 52–57 https://doi.org/10.1111/j.1440-1746.2011.07009.x

72 Mishra, S., Saleh, A., Espino, P.S., Davie, J.R. and Murphy, L.J. (2006) Phosphorylation of histones by tissue transglutaminase. *J. Biol. Chem.* **281**, 5532–5538 https://doi.org/10.1074/jbc.M506864200

73 Lee, C.S. and Park, H.H. (2017) Structural aspects of transglutaminase 2: functional, structural, and regulatory diversity. *Apoptosis* **22**, 1057–1068 https://doi.org/10.1007/s10495-017-1396-9

74 Phatak, V.M., Croft, S.M., Rameshaiah Setty, S.G., Scarpellini, A., Hughes, D.C., Rees, R. et al. (2013) Expression of transglutaminase-2 isoforms in normal human tissues and cancer cell lines: dysregulation of alternative splicing in cancer. *Amino Acids* **44**, 33–44 https://doi.org/10.1007/s00726-011-1127-4

75 Ai, L., Kim, W.-J., Demircan, B., Dyer, L.M., Bray, K.J., Skehan, R.R. et al. (2008) The transglutaminase 2 gene (TGM2), a potential molecular marker for chemotherapeutic drug sensitivity, is epigenetically silenced in breast cancer. *Carcinogenesis* **29**, 510–518 https://doi.org/10.1093/carcin/bgm280

76 Mehta, K., Fok, J., Miller, F.R., Koul, D. and Sahin, A.A. (2004) Prognostic significance of tissue transglutaminase in drug resistant and metastatic breast cancer. *Clin. Cancer Res.* **10**, 8068–8076 https://doi.org/10.1158/1078-0432.CCR-04-1107

77 Minotti, L., Baldassari, F., Galasso, M., Volinia, S., Bergamini, C.M. and Bianchi, N. (2018) A long non-coding RNA inside the type 2 transglutaminase gene tightly correlates with the expression of its transcriptional variants. *Amino Acids* **50**, 421–438 https://doi.org/10.1007/s00726-017-2528-9

78 Zonca, S., Pinton, G., Wang, Z., Soluri, M.F., Tavian, D., Griffin, M. et al. (2017) Tissue transglutaminase (TG2) enables survival of human malignant pleural mesothelioma cells in hypoxia. *Cell Death Dis.* **8**, e2592 https://doi.org/10.1038/cddis.2017.30

79 He, W., Sun, Z. and Liu, Z. (2015) Silencing of TGM2 reverses epithelial to mesenchymal transition and modulates the chemosensitivity of breast cancer to docetaxel. *Exp. Ther. Med.* **10**, 1413–1418 https://doi.org/10.3892/etm.2015.2679

80 Hwang, J.Y., Mangala, L.S., Fok, J.Y., Lin, Y.G., Merritt, W.M., Spannuth, W.A. et al. (2008) Clinical and biological significance of tissue transglutaminase in ovarian carcinoma. *Cancer Res.* **68**, 5849–5858 https://doi.org/10.1158/0008-5472.CAN-07-6130

81 Shao, M., Cao, L., Shen, C., Satpathy, M., Chelladurai, B., Bigsby, R.M. et al. (2009) Epithelial-to-mesenchymal transition and ovarian tumor progression induced by tissue transglutaminase. *Cancer Res.* **69**, 9192–9201 https://doi.org/10.1158/0008-5472.CAN-09-1257

82 Oh, K., Lee, O.-Y., Park, Y., Seo, M.W. and Lee, D.-S. (2016) IL-1β induces IL-6 production and increases invasiveness and estrogen-independent growth in a TG2-dependent manner in human breast cancer cells. *BMC Cancer* **16**, 724 https://doi.org/10.1186/s12885-016-2746-7

83 Mann, A.P., Verma, A., Sethi, G., Manavathi, B., Wang, H., Fok, J.Y. et al. (2006) Overexpression of tissue transglutaminase leads to constitutive activation of nuclear factor-κB in cancer cells: delineation of a novel pathway. *Cancer Res.* **66**, 8788–8795 https://doi.org/10.1158/0008-5472.CAN-06-1457

84 Park, S.-S., Kim, J.-M., Kim, D.-S., Kim, I.-H. and Kim, S.-Y. (2006) Transglutaminase 2 mediates polymer formation of I-κBα through C-terminal glutamine cluster. *J. Biol. Chem.* **281**, 34965–34972 https://doi.org/10.1074/jbc.M604150200

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85 Brown, K.D. (2013) Transglutaminase 2 and NF-κB: an odd couple that shapes breast cancer phenotype. *Breast Cancer Res. Treat.* **137**, 329–336 https://doi.org/10.1007/s10549-012-2351-7
86 Caccamo, D., Campisi, A., Curro, M., Aguennouz, M., Volti, G.L., Avola, R. et al. (2005) Nuclear factor-κB activation is associated with glutamate-evoked tissue transglutaminase upregulation in primary astrocyte cultures. *J. Neurosci. Res.* **82**, 858–865 https://doi.org/10.1002/jnr.20683
87 Kumar, S. and Mehta, K. (2012) Tissue transglutaminase constitutively activates HIF-1α promoter and nuclear factor-κB via a non-canonical pathway. *PLoS ONE* **7**, e49321 https://doi.org/10.1371/journal.pone.0049321
88 Huang, L., Haylor, J.L., Fisher, M., Hau, Z., El Nahas, A.M., Griffin, M. et al. (2010) Do changes in transglutaminase activity alter latent transforming growth factor beta activation in experimental diabetic nephropathy? *Nephrol. Dial. Transplant.* **25**, 3897–3910 https://doi.org/10.1093/ndt/gfq291
89 Eom, S., Kim, Y., Kim, M., Park, D., Lee, H., Lee, Y.S. et al. (2014) Transglutaminase II/microRNA-218/-181a loop regulates positive feedback relationship between allergic inflammation and tumor metastasis. *J. Biol. Chem.* **289**, 29483–29505 https://doi.org/10.1074/jbc.M114.603480
90 Cellura, D., Pickard, K., Quaratino, S., Parker, H., Strefford, J.C., Thomas, G.J. et al. (2015) miR-19-mediated inhibition of transglutaminase-2 leads to enhanced invasion and metastasis in colorectal cancer. *Mol. Cancer Res.* **13**, 1095–1105 https://doi.org/10.1158/1541-7786.MCR-14-0466
91 Hidaka, H., Seki, N., Yoshino, H., Yamasaki, T., Yamada, Y., Nohata, N. et al. (2012) Tumor suppressive microRNA-1285 regulates novel molecular targets: aberrant expression and functional significance in renal cell carcinoma. *Oncotarget* **3**, 44–57 https://doi.org/10.18632/oncotarget.417
92 Korde, A., Jin, L., Zhang, J.-G., Ramaswamy, A., Hu, B., Kolahian, S. et al. (2017) Lung endothelial microRNA-1 regulates tumor growth and angiogenesis. *Am. J. Respir. Crit. Care Med.* **196**, 1443–1455 https://doi.org/10.1164/rccm.201610-2157OC
93 Qu, W., Chen, X., Wang, J., Lv, J. and Yan, D. (2017) MicroRNA-1 inhibits ovarian cancer cell proliferation and migration through c-Met pathway. *Clin. Chim. Acta* **473**, 237–244 https://doi.org/10.1016/j.cca.2017.07.008
94 Xie, M., Dart, D.A., Guo, T., Xing, X.-F., Cheng, X.-J., Du, H. et al. (2018) MicroRNA-1 acts as a tumor suppressor microRNA by inhibiting angiogenesis-related growth factors in human gastric cancer. *Gastric Cancer* **21**, 41–54 https://doi.org/10.1007/s10120-017-0721-x
95 Ahrend, H., Kaul, A., Ziegler, S., Brandenburg, L.-O., Zimmermann, U., Mustea, A. et al. (2017) MicroRNA-1 and microRNA-21 individually regulate cellular growth of non-malignant and malignant renal cells. *In Vivo* **31**, 625–630 https://doi.org/10.21873/invivo.11103
96 Xu, L., Zhang, W., Sun, R., Liu, J., Hong, J., Li, Q. et al. (2017) IGF-1 may predict the severity and outcome of patients with sepsis and be associated with microRNA-1 level changes. *Exp. Ther. Med.* **14**, 797–804 https://doi.org/10.3892/etm.2017.4553
97 Kang, B., Li, W., Xi, W., Yi, Y., Ciren, Y., Shen, H. et al. (2017) Hydrogen sulfide protects cardiomyocytes against apoptosis in ischemia/reperfusion through miR-1-regulated histone deacetylase 4 pathway. *Cell. Physiol. Biochem.* **41**, 10–21 https://doi.org/10.1159/000455816
98 Jin, P., Gu, W., Lai, Y., Zheng, W., Zhou, Q. and Wu, X. (2017) The circulating microRNA-206 level predicts the severity of pulmonary hypertension in patients with left heart diseases. *Cell. Physiol. Biochem.* **41**, 2150–2160 https://doi.org/10.1159/000475569
99 Wang, M., Ji, Y., Cai, S. and Ding, W. (2016) MiR-206 suppresses the progression of coronary artery disease by modulating vascular endothelial growth factor (VEGF) expression. *Med. Sci. Monit.* **22**, 5011–5020 https://doi.org/10.12659/MSM.898883
100 Wu, H., Tao, J., Li, X., Zhang, T., Zhao, L., Wang, Y. et al. (2017) MicroRNA-206 prevents the pathogenesis of hepatocellular carcinoma by modulating expression of cMet and Cdk6. *Hepatology* **66**, 1952–1967 https://doi.org/10.1002/hep.29374
101 Wu, H., Zhang, T., Pan, F., Steer, C.J., Li, Z., Chen, X. et al. (2017) MicroRNA-206 prevents hepatosteatosis and hyperglycemia by facilitating insulin signaling and impairing lipogenesis. *J. Hepatol.* **66**, 816–824 https://doi.org/10.1016/j.jhep.2016.12.016
102 Hou, C.-G., Luo, X.-Y. and Li, G. (2016) Diagnostic and prognostic value of serum microRNA-206 in patients with gastric cancer. *Cell. Physiol. Biochem.* **39**, 1512–1520 https://doi.org/10.1159/000447854
103 Fu, X., Cui, Y., Yang, S., Xu, Y. and Zhang, Z. (2016) MicroRNA-613 inhibited ovarian cancer cell proliferation and invasion by regulating KRAS. *Tumour Biol.* **37**, 6477–6483 https://doi.org/10.1007/s13277-015-4507-7
104 Lu, Y., Tang, L., Zhang, Q., Zhang, Z. and Wei, W. (2017) MicroRNA-613 inhibits the progression of gastric cancer by targeting CDK9. *Artif. Cells Nanomed. Biotechnol.* **45**, 1–5 https://doi.org/10.1080/21691401.2017.1351983
105 Zhao, R., Feng, J. and He, G. (2014) MiR-613 regulates cholesterol efflux by targeting LXRα and ABCA1 in PPARγ activated THP-1 macrophages. *Biochem. Biophys. Res. Commun.* **448**, 329–334 https://doi.org/10.1016/j.bbrc.2014.04.052
106 Di, G., Wang, Z., Wang, W., Cheng, F. and Liu, H. (2017) AntagomiR-613 protects neuronal cells from oxygen glucose deprivation/re-oxygenation via increasing SphK2 expression. *Biochem. Biophys. Res Commun.* **493**, 188–194 https://doi.org/10.1016/j.bbrc.2017.09.049
107 Fagerberg, L., Hallström, B.M., Oksvold, P., Kampf, C., Djureinovic, D., Odeberg, J. et al. (2014) Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. *Mol. Cell. Proteomics* **13**, 397–406 https://doi.org/10.1074/mcp.M113.035600
108 Kerr, C., Szmacinski, H., Fisher, M.L., Nance, B., Lakowicz, J.R., Akbar, A. et al. (2017) Transamidase site-targeted agents alter the conformation of the transglutaminase cancer stem cell survival protein to reduce GTP binding activity and cancer stem cell survival. *Oncogene* **36**, 2981–2990 https://doi.org/10.1038/onc.2016.452
109 Boroughs, L.K., Antonyak, M.A. and Cerione, R.A. (2014) A novel mechanism by which tissue transglutaminase activates signaling events that promote cell survival. *J. Biol. Chem.* **289**, 10115–10125 https://doi.org/10.1074/jbc.M113.464693
110 Ozpolat, B., Akar, U., Mehta, K. and Lopez-Berestein, G. (2007) PKCδ and tissue transglutaminase are novel inhibitors of autophagy in pancreatic cancer cells. *Autophagy* **3**, 480–483 https://doi.org/10.4161/auto.4349
111 Festoff, B.W., SantaCruz, K., Arnold, P.M., Sebastian, C.T., Davies, P.J.A. and Citron, B.A. (2002) Injury-induced 'switch' from GTP-regulated to novel GTP-independent isoform of tissue transglutaminase in the rat spinal cord. *J. Neurochem.* **81**, 708–718 https://doi.org/10.1046/j.1471-4159.2002.00850.x
112 De Laurenzi, V. and Melino, G. (2001) Gene disruption of tissue transglutaminase. *Mol. Cell. Biol.* **21**, 148–155 https://doi.org/10.1128/MCB.21.1.148-155.2001
113 Nanda, N., Iismaa, S.E., Owens, W.A., Husain, A., Mackay, F. and Graham, R.M. (2001) Targeted inactivation of G_h/tissue transglutaminase II. *J. Biol. Chem.* **276**, 20673–20678 https://doi.org/10.1074/jbc.M010846200
114 Kárpáti, S., Sárdy, M., Németh, K., Mayer, B., Smyth, N., Paulsson, M. et al. (2018) Transglutaminases in autoimmune and inherited skin diseases: the phenomena of epitope spreading and functional compensation. *Exp. Dermatol.* **71**, 402 https://doi.org/10.1111/exd.13449

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