Possible roles of transglutaminases in molecular mechanisms responsible for cancer and human neurodegenerative diseases

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Abstract

Transglutaminases are a family of Ca²⁺-dependent enzymes which catalyze post-translational modifications of proteins. The main activity of these enzymes is the cross-linking of glutaminyl residues of a protein/polypeptide substrate to lysyl residues of a protein/polypeptide co-substrate. In addition to lysyl residues, other second nucleophilic co-substrates may include monoamines or polyamines (to form mono- or bi-substituted/crosslinked adducts) or -OH groups (to form ester linkages). In absence of co-substrates, the nucleophile may be water, resulting in the net deamidation of the glutaminyl residue. Transglutaminase activity has been suggested to be involved in molecular mechanisms responsible for either physiological or pathological processes. In particular, transglutaminase activity has been shown to be responsible for human autoimmune diseases and Celiac Disease is just one of them. Interestingly, cancer and neurodegenerative diseases, such as Alzheimer’s Disease, Parkinson’s Disease, supranuclear palsy and Huntingdon’s Disease, are characterized in part by aberrant transglutaminase activity and by increased cross-linked proteins in affected tissues. This review describes the possible molecular mechanisms by which these enzymes could be responsible for such diseases and the possible use of transglutaminase inhibitors for patients with diseases characterized by aberrant transglutaminase activity.

Biochemistry of the transglutaminases

Transglutaminases (TGs, E.C. 2.3.2.13) are family of Ca²⁺-dependent enzymes which catalyze post-translational modifications of proteins. Examples of TG-catalyzed reactions include: I) acyl transfer between the γ-carboxamide group of a protein/polypeptide glutaminyl residue and the ε-amino group of a protein/polypeptide lysyl residue; II) attachment of a polyanine to the γ-carboxamide of a glutaminyl residue; III) deamination of the γ-carboxamide group of a protein/polypeptide glutaminyl residue.¹,² The reactions catalyzed by TGs occur by a two-step mechanism (ping-pong type). The transamidating activity of TGs is activated by the binding of Ca²⁺, which exposes an active-site cysteine residue. This cysteine residue reacts with the γ-carboxamide group of an incoming glutaminyl residue of a protein/polypeptide substrate to yield a thioacetyl-enzyme intermediate and ammonia. The thioaoyl-enzyme intermediate then reacts with a nucleophilic primary amine substrate, resulting in the covalent attachment of the amine-containing donor to the substrate glutaminyl acceptor and regeneration of the cysteinyl residue at the active site. If the primary amine is donated by the ε-amino group of a lysyl residue in a protein/polypeptide, a Nε-(γ-L-glutamyl)-L-lysine (Gigel) isopeptide bond is formed. On the other hand, if a polyanine or another primary amine (e.g. histamine, serotonin and others) acts as the amine donor, a γ-glutamylpolyamine (or γ-glutamylamine) residue is formed. It is also possible for a polyanine to act as an N,N-bis-(γ-L-glutamyl)polyamine bridge between two glutaminyl acceptor residues either on the same protein/polypeptide or between two proteins/polypeptides.³ If there is no primary amine present, water may act as the attacking nucleophile, resulting in the deamination of glutaminyl residues to glutamyl residues. Regarding the physiological roles played by the transglutaminase activity, recently transglutaminase-catalyzed polyamination of tubulin has been shown to stabilize axonal microtubules, suggesting an important role for these reactions also during some physiological processes, such as neurite outgrowth and axon maturation.⁴ The reactions catalyzed by TGs occur with little change in free energy and hence should theoretically be reversible. However, under physiological conditions the cross-linking reactions catalyzed by TGs are usually irreversible. This irreversibility partly results from the metabolic removal of ammonia from the system and from thermodynamic considerations resulting from altered protein conformation. Some scientific reports suggest that TGs may be able to catalyze the hydrolysis of Gigel cross links isopeptide bonds in some soluble cross-linked proteins. Furthermore, it is likely that TGs can catalyze the exchange of polyamines onto proteins.⁵ In TG2 other catalytic activities, such as the ability to hydrolyze GTP (or ATP) into GDP (or ADP) and inorganic phosphate, a protein disulfide isomerase activity and a kinase activity which phosphorylates histones, retinoblastoma (RB) and P53 are present, while only some of these activities have been identified also in other TGs.⁶,⁷

Ample experimental evidence indicate that some TGs are multifunctional proteins with distinct and regulated enzymatic activities. In fact, under physiological conditions, the transamidation activity of TGs is latent,⁸,⁹ while other activities, recently identified, could be present. For example, in some physiological states, when the concentration of Ca²⁺ increases, the crosslinking activity of TGs may contribute to important biological processes. As previously described, one of the most intriguing

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Key words: Transglutaminases; Post-translational modifications of proteins; Cancer; NF-kB; Neurodegeneration.

Contributions: N.G.G., G.C., M.R.Z. and A.G. selected studies and collected data from literature; V.G., and G.M. prepared the manuscript; N.G.G., G.C., M.R.Z. and A.G. implemented and edited the manuscript; V.G. and G.M. critically revised the manuscript; V.G. finally approved the manuscript.

Funding: this work is supported by the Italian Education Department and the Campania Region (L.R. n.5 del 28.03.2002, finanziamento 2008) entitled: Identificazione e caratterizzazione di geni della transglutaminasi nel Sistema Nervoso in relazione allo sviluppo di malattie neurodegenerative (Identification and characterization of transglutaminase genes in the Nervous System in relationship to the development of neurodegenerative diseases).

Conflict of interest: the authors declare no potential conflict of interest.

Received for publication: 26 January 2017. Accepted for publication: 24 February 2017.

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properties of some TGs, such as TG2, is the ability to bind and hydrolyze GTP and furthermore, to bind to GTP and Ca²⁺. GTP and Ca²⁺ regulate its enzymatic activities, including protein cross-linking, in a reciprocal manner: the binding of Ca²⁺ inhibits GTP-binding and GTP-binding inhibits the transglutaminase cross-linking activity of the TG2. Interestingly, TG2 shows no sequence homology with heterotrimeric or low-molecular-weight G-proteins, but there is evidence that TG2 (TG2/Ghα) is involved in signal transduction, and, therefore, TG2/Ghα should also be classified as a large molecular weight G-protein. Other studies, along with ours, showed that TG2/Ghα can mediate the activation of phospholipase C (PLC) by the α₄-adrenergic receptor and can modulate adenyl cyclase activity. TG2/Ghα can also mediate the activation of the 61 isoform of PLC and of maxi-K channels. Interestingly, the signaling function of TG2/Ghα is preserved even with the mutagenic inactivation of its crosslinking activity by the mutation of the active site cysteine residue.

Transglutaminases and signal transduction

Beside the well-known function as a crosslinking enzyme, TG2 is able to interact with different target proteins dislocated in extracellular matrix, membrane, cytoplasm, mitochondrion and nucleus. This fact implies that TG2 can work as GTPase/ATPase, non-enzymatic adapter, scaffold protein or signal transduction regulator in processes such as transamidation and protein-protein crosslinking. Among the most characterized transglutaminase-regulated cell signaling there is the pathway of TGF-β, whose maturation and activity is induced by TG2 through a covalent crosslink with latent TGF-β1 binding protein. Change in growth factor activity results in extracellular environment remodeling involving integrins and proteases. Furthermore, in tumor models, TG2 expression was reported to be induced by TG2-GTP, resulting in the induction of epithelial-to-mesenchymal transition (EMT), an embryonic process that can be reactivated in adult tissues in response to epigenetic changes. In turn, a mechanism involving NF-kB pathway is responsible for the increase, in fibroblasts, of both TGF-β mRNA and protein expression. Therefore TG2 and TGF-β are reciprocally regulated in a positive feedback loop. TG2 acts, in fact, also as a NF-kB activator in a IKK-independent mechanism that involves TG2 crosslinking of IκBα with subsequent IκBα degradation via a non-proteasomal mechanism. TG2 also promotes polymerization of IκBα that induces IκBα proteasomal degradation. Both mechanisms lead to NF-kB activation. In addition, TG2 contributes to cell attachment to the matrix, activating integrin signaling that is achieved by TG2 direct binding with β1, β3 and β5 integrins. A strong interaction also affects TG2 and fibronectin. Whereby it occurs a dual binding both with fibronectin and integrin, that potentiates integrin/fibronectin interaction. More recently, it has been identified a role for TG2 in the interplay between integrin and platelet-derived growth factor receptor (PDGFR). This interaction enhances PDGFR binding with integrins and thus upregulating growth factor receptor downstream signalling and PDGFR turnover. It results in the stimulation of PDGF/PDGFR-induced Akt1 and Shp2 activation in fibroblasts and vascular smooth muscle cells, enhancing cell proliferation, survival and migration. TG2 also affects VEGF pathway by covalently binding VEGFR on the surface of endothelial cells, so originating a high-molecular-weight complex that heads toward the nucleus and potentiates VEGF-induced ERK activation. Also EGFR pathway, that is commonly aberrantly activated in cancer, induces the expression of TG2 in epithelial tumor cells; this is in direct correlation with the increase of TG2-dependent transamidation and with the subsequent activation EGF-dependent signalling involving Ras, PI3K, JNK, ERK and NF-kB pathways. As a result of EGF-mediated TG2 induction, it can be observed enhanced migration, invasiveness and anchorage-independent cancer cell growth.

Of note, TG2 mediates monoaminylation of cytoplasmic proteins regulating signaling and vesicular trafficking; among these post-translational modifications, there is the serotonylation of RhoA and Rab4A GTPases, essential for cytoskeletal reorganization and subsequent exocytosis of platelet α-granules, platelet activation, platelet adhesion, and platelet aggregation. RhoA activation, through TG2-mediated monoaminylation, is also correlated with Akt1 activation. Another post-translational modification triggered by transglutaminase is the eIF5A-1 hypusination, as demonstrated by the in vitro formation of a γ-glutamyl-hypusine bond among eIF5A-1 and dimethylcasein. This event was put in correlation with the inhibition of cell proliferation and apoptosis induction. On the other hand, it was reported that IFNα reduced the hypusination and activation of eIF5A-1 in parallel with the induction of growth inhibition in human epithemoid cancer KB cells and all these effects were antagonized by EGF. Concerning type I IFN, and particularly IFNβ, it was reported that it can stimulate the transcription of TG2, although the precise mechanism has not been wholly elucidated. Moreover, IFN can also affect TG intracellular stability acting on its ubiquitination, maybe through the induction of two ubiquitin cross-reactive enzymes: UbcH5 and UbcH8. Similarly, also IFNs have been demonstrated stimulatory activity over TG2 expression in a global anticancer mechanism that involves the inhibition, IFNα-mediated, of TG2 degradation via the ubiquitin proteasome system. Regardless of transamidating activity, TG2 also interacts with GTP and acts as GPase, in spite of the lack of four consensus GTP-binding motifs common to the classical G proteins. The catalytic rate is comparable to the α subunits of G

Molecular biology of the transglutaminases

To date at least eight different TGs, distributed in the human body, have been identified (Table 1). Complex gene expression mechanisms regulate the physiological roles that these enzymes play in both the intracellular and extracellular compartments. In the Nervous System, for example, several forms of TGs are simultaneously expressed. Moreover, in these last years, several alternative splice variants of TGs, mostly in the 3'-end region, have been identified. Interestingly, some of them are differently expressed in human pathologies, such as Alzheimer’s Disease (AD). On the basis of their ubiquitous expression and their biological roles, we may speculate that the absence of these enzymes would be lethal. However, this does not always seem to be the case, since, for example, null mutants of the TG2 are usually phenotypically normal at birth. This result may be explained by the expression of other TG genes that may substitute the TG2 missing isoform, although other TG isoform mutations have been associated with severe phenotypes, such as lamellar ichthyosis for TG1 isoform mutations. Bioinformatic studies have shown that the primary structures of human TGs share some identities in only few regions, such as the active site and the calcium binding regions. However, high sequence conservation and, therefore, a high degree of preservation of secondary structure among TG2, TG3 and FXIIa indicate that these TGs all share four-domain tertiary structures which could be similar to those of other TGs.
proteins and mutating Arg580 accounts for an about 100-fold decrease in GTP-binding affinity and deletes the inhibitory activity of GTP against TG2-mediated transamidation.47 TG2-mediated protein crosslinking, transamidation and GTPase activity are also realized in nuclear compartment, where it is localized an about 5% of TG2 total cellular amount.48 Nuclear TG2 is involved in chromatin binding and, moreover, in the regulation of gene expression through the transamidation of both histones and transcription factors, such as Sp1, which is inactivated by TG2 crosslink, with subsequent downregulation of growth factor receptors, including e-Met, and cell death.49 Finally, TG2 is also present in both mitochondrial membrane and mitochondrial matrix;49,50 a sequence homology with BH3 domain of Bcl-2 mitochondrial proteins, indicates an apoptotic role51 that can be pro- or anti-apoptotic, depending on the cell type and on the precise death stimulus. For instance, TG2 can crosslink the proapoptotic members inducing cell death in certain cell models and, on the contrary, it can inhibits calcium-induced apoptosis in other cell types.51 Moreover, several reports have also shown TG2-induced transamidation and crosslinking of mitochondrial proteins, although it seems that these modifications are a peculiarity of not normal tissues, such as the ones affected by mitochondrial diseases, i.e. those suffering from cardiovascular or neurodegenerative disorders.52 Overall, these results support the hypothesis that increased TG2 expression levels are able to promote proliferative pathways both by its binding with key signaling proteins and by acting on the extracellular matrix.

Transglutaminases and cancer

Among the best characterized physiological roles of TG2 there is the regulation of several processes, such as cell growth and differentiation,53 tumor metastasis and programmed cell death.54,55 Aside from genetic and epigenetic changes, that can be induced by several stressor agents, a strong contribute in dysregulating cell growth and differentiation seems to be due to chronic inflammation. It gives, in fact, a further effort in conferring metastatic potential and capability to survive in stromal environment, thus contributing to cancer initiation and progression.56 Assuming that TG2 is commonly upregulated in inflamed tissues,57 it is expected that TG2 could be involved in cancer development, although the molecular mechanisms remain mostly to be delucidated. Anyway, it has been demonstrated that TG2 expression is low in primary tumors and higher in drug-resistant cancers and metastatic tissues.58,59 The correlation between TG2 and inflammation that, on the basis of what has just been stated, establish an indirect correlation between TG2 and cancer, has been documented by the fact that the common inflammatory cytokines (TGF-β, TNF-α, IL-1, and IL-6) are well-known TG2 stimulators60,61 and are able to establish an active autocrine stimulation in tumor environment. Cytokine-induced increase of TG2 levels stimulates both synthesis and deposition of fibronectin and collagen; simultaneously, extracellular TG2 crosslinks them and stabilizes the extracellular matrix, thereby inducing tumor desmoplastic response62 and promoting malignant phenotype. As previously stated, TG2-dependent activation of NF-κB stimulation, as well as also FAK and Akt stimulation, make it a strong EMT inducer.63,64 EMT controls cell behavior during cancer progression in an effort to hold inflammation and repair damaged tissues; nevertheless, in pathological tissues this response can account for damage, resulting in intravasation, extravasation and micrometastases formation and the acquisition of stem-cell phenotype.63,65,66 Furthermore, hypoxia-response transcription factor HIF-1, whose expression is controlled by NF-κB67 and TG2,63,65,66 has itself an important role in cancer development and in inflammatory signaling regulation, controlling EMT, stemness, drug resistance and metastatic phenotype. In fact, cells expressing high TG2 levels exhibit elevated HIF-1α levels, even under normoxic conditions; on the other hand, enforced inhibition of either TG2 or NF-κB induces a decrease of HIF-1α expression.68 Of note, the covalent modification of basement membrane proteins (laminin, collagen and diverse types of extracellular matrix proteins) by TG may interfere with the adhesiveness and invasiveness of tumor cells,69,70 thus further supporting the participation of this enzyme in the migration and adhesion processes occurring during the metastatic spread of cancer cells. TG2 catalyzes the incorporation of spermidine into the components of basal membrane, in a process that generally involves FAD-dependent polyamine oxidase (PAO)71 and that may be one of the cellular mechanisms regulating the preferential formation of a sterically defined bis(γ-glutamyl)spermidine cross-link. In virtue of this fact, it was investigated the effect of this catalytic activity on the adhesion and invasion capability of murine B16-F10 melanoma cells. The analysis was carried out using lamine or matrigel as reference components of basal membrane. In virtue of the impairment of extracellular matrix crosslinking, adhesiveness of B16-F10 cells to basal membrane proteins become reduced when the basal-like proteins were modified by spermidine, due to TG2 catalysis. This is of interest taking into account that the adhesion of circulating tumor cells to endothelium and their transition through the vascular endothelium is a main stage in cancer invasion and metastasis.72,73 Concerning the previously hinted TG2 involvement in drug resistance, a recent report has described the correlation between TG2 and human MCF-7 breast cancer cells’ resistance to vorinostat, as well as to other histone deacetylase (HDAC) inhibitors. TG2 mRNA and protein expression levels, along with TG2 transamidating activity, were paralleled with vorinostat-induced anti-proliferative effects, founding an inverse correlation and suggesting that TG2 could represent a mechanism of intrinsic or acquired resistance to vorinostat.74 Similar results have also been observed for MCF-7 cells treated with doxorubicin, where there was a selection of a small cell subpopulation with stemness characteristics and high TG2 expression levels.75 All these findings strongly suggest that TG2 can promote the EMT and is also responsible for the induction of a stem cell behaviour in tumor cells.

Transglutaminases as target for therapeutic intervention in cancer

Drug resistance and metastasization are features closely related to an advanced stage (relapsed or refractory) of cancer and represent the most clinical obstacle to the success of anti-cancer treatment, accounting for about 90% of cancer-related deaths. The discovery of tumor-associated genes able to induce metastasization and resistance to anti-cancer therapy is an imperative objective in order to properly target them and to employ the corresponding proteins as cancer biomarkers. Based on the pleiotropic characteristics of TG2 and on the finding of higher TG2 expression levels in tumor samples, compared to the normal counterpart,76-78 it was widely investigated the role played by TG2 in inducing cancer hallmarks. TG2 inhibition, employing siRNA, antisense RNA or small molecule inhibitors, should represent a promising therapeutic option for treating advanced cancers and counteracting entire inflammatory networks (NF-κB, AKT, FAK, HIF-1α, etc.) that play a crucial role in inducing angiogenesis, drug resistance, metastasization and EMT. Targeted thera-

[Translational Medicine Reports 2017; 1:6599]
pies affecting a single gene or pathway are often responsible for the occurrence of resistance, therefore it is widely desirable an anti-cancer strategy based on the inhibition of multiple signalling pathways that work in parallel in cancer cells. This can be achieved either by multi-drug therapy or by multi-targeting agents, such as TG2 inhibitors. To date, several small TG2 inhibitors have been developed. However, if on one hand some of these have shown promising in vitro and in vivo activity, their clinical use still presents serious limitations. TGs are complex proteins with various structural domains bearing different functions; therefore, it is difficult to target them using small molecules or antibodies that, among other things, often lack specificity and may originate undesirable side effects. In this regard, siRNA treatment to inhibit TG2 expression remains the most useful approach for cancer cells treatment. Liposomal delivery of siRNA has been successfully employed in orthotopically growing tumors, showing a significant downregulation of TG2 expression. This delivery strategy resulted in in vivo TG2 silencing as well as in the inhibition of growth and dissemination of pancreatic and ovarian cancer cells, also rendering them sensitive to chemotherapeutic drugs in a nude mouse model. These results endorse the hypothesis that high TG2 levels may not only be used as reliable prognostic markers for early tumor diagnosis but also as promising therapeutic target for treating advanced tumors.

Role of the transglutaminases in neurodegenerative diseases

Although numerous scientific reports suggest that the transglutaminase activity is involved in the pathogenesis of neurodegenerative diseases, to date, however, still controversial experimental findings about the role of the TGs enzymes in these diseases have been obtained. Protein aggregates in affected brain regions are histopathological hallmarks of many neurodegenerative diseases. More than 20 years ago Selkoe et al. suggested that TG activity might contribute to the formation of protein aggregates in AD brain. In support of this hypothesis, tau protein has been shown to be an excellent in vitro substrate of TGs and GGEI cross-links have been found in the neurofibrillary tangles and paired helical filaments of AD brains. Interestingly, a recent work showed the presence of by glutamyl putrescine in human CSF, which was increased in Huntington’s Disease (HD) CSF. This is an important evidence that protein/peptides crosslinking by polyamines does indeed occur in the brain, and that this is increased in HD brain. TG activity has been shown to induce also amyloid β-protein oligomerization and aggregation at physiologic levels. By these molecular mechanisms, TGs could contribute to AD symptoms and progression. Moreover, there is evidence that TGs also contribute to the formation of proteinaceous deposits in Parkinson’s Disease (PD), in supranuclear palsy and in HD, a neurodegenerative disease caused by a CAG expansion in the affected gene. For example, expanded polyglutamine domains have been reported to be substrates of TG2 and therefore aberrant TG activity could contribute to CAG-expansion diseases, including HD. However, although all these studies suggest the possible involvement of the TGs in the formation of deposits of protein aggregates in neurodegenerative diseases, they do not indicate whether aberrant TG activity per se directly determines the disease progression. For example, several experimental findings reported that TG2 activity in vitro leads to the formation of soluble aggregates of α-synuclein or polyQ proteins. To date, as previously reported, at least ten human CAG-expansion diseases have been described (Table 2) and in at least eight of them their neuropathology is caused by the expansion in the number of residues in the polyglutamine domain to a value beyond 35-40. Remarkably, the mutated proteins have no obvious similarities except for the expanded polyglutamine domain. In fact, in all cases except SCA 12, the mutation occurs in the coding region of the gene. However, in SCA12, the CAG triplet expansion occurs in the untranslated region at the 5’ end of the PPP2R2B gene. It has been proposed that the toxicity results from overexpression of the brain specific regulatory subunit of protein phosphatase PP2A. Most of the mutated proteins are widely expressed both within the brain and elsewhere in the body. A major challenge then is to understand why the brain is primarily affected and why different regions within the brain are affected in the different CAG-expansion diseases, i.e., what accounts for the neurotoxic gain of function of each protein and for a selective vulnerability of each cell type. Possibly, the selective vulnerability may be explained in part by the susceptibility of the expanded poliglutamine domains in the various CAG-expansion diseases to act as cosubstrates for a brain TG. To strengthen the possible central role of the TGs in neurodegenerative diseases, a study by Hajivassiliou et al. showed that anti-TG2 IgA antibodies are present in the gut and brain of patients with gluten ataxia, a non-genetic sporadic cerebellar ataxia, but not in ataxia control patients. Recently, anti-TG2, -TG3 and -TG6 antibodies have been found in sera from CD patients, suggesting a possible involvement also of other TGs in the pathogenesis of dermatitis herpetiformis and gluten ataxia, two frequent extra-intestinal manifestations of gluten sensitivity. These last findings could support also a possible role of the gut-brain axis for the etiopathogenesis of human neurodegenerative diseases, in which the TG enzymes, in particular the TG2 enzyme, could play an important role.

In support of the hypothesis of the toxic effect of TG activity in other neurodegenerative diseases, such as Alzheimer’s disease and Parkinson’s Disease, TG activity has been shown to induce amyloid beta-protein and α-synuclein oligomerization and aggre-

<table>
<thead>
<tr>
<th>TG</th>
<th>Physiological role</th>
<th>Gene map location</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor XIII</td>
<td>Blood clotting</td>
<td>6p24.25</td>
<td>14</td>
</tr>
<tr>
<td>TG 1 (Keratinocyte TG, kTG)</td>
<td>Skin differentiation</td>
<td>14q11.2</td>
<td>15</td>
</tr>
<tr>
<td>TG 2 (Tissue TG, ITG, cTG)</td>
<td>Apoptosis, cell adhesion, signal transduction</td>
<td>20q11.2</td>
<td>16</td>
</tr>
<tr>
<td>TG 3 (Epidermal TG, eTG)</td>
<td>Hair follicle differentiation</td>
<td>20p11.2</td>
<td>17</td>
</tr>
<tr>
<td>TG 4 (Prostate TG, pTG)</td>
<td>Suppression of sperm immunogenicity</td>
<td>3q21.2</td>
<td>18</td>
</tr>
<tr>
<td>TG 5 (TG X)</td>
<td>Epidermal differentiation</td>
<td>15q15.2</td>
<td>19</td>
</tr>
<tr>
<td>TG 6 (TG Y)</td>
<td>Central Nervous System development</td>
<td>20p13</td>
<td>19</td>
</tr>
<tr>
<td>TG 7 (TG Z)</td>
<td>Unknown function</td>
<td>15q15.2</td>
<td>19</td>
</tr>
</tbody>
</table>
In fact, TG activity induces proteofibril-like amyloid beta-protein assemblies that are protease-resistant and inhibit long-term potentiation. Therefore, by these molecular mechanisms, TG activity could also contribute to Alzheimer’s disease symptoms and progression. Very recently, TG2 and its isopeptide product have been found increased in Alzheimer’s disease and APPswe/PS1dE9 double transgenic mice brains, while catalytically active TG2 colocalizes with Aβ pathology in Alzheimer’s disease mouse models. Interestingly, other works are suggesting that also other TGs could be involved in the molecular mechanisms responsible for neurodegenerative diseases. In particular, a recent work by Basso et al. found that in addition to TG2, TG1 gene expression level is significantly induced following stroke in vivo or due to oxidative stress in vitro. Moreover, structurally diverse inhibitors, used at concentrations that simultaneously inhibit TG1 and TG2 are neuroprotective. Together, these last studies suggested that multiple TG isoforms, not only TG2, participate in oxidative stress-induced cell death signalling, and that isoform nonspecific inhibitors of TG will be most efficacious in combating oxidative death in neurological disorders. These are interesting and worthwhile studies, suggesting that multiple TG isoforms can participate in neuronal death processes. Therefore, all these studies suggest that the involvement of brain TGs could represent a common denominator in several neurological diseases, which can lead to the determination of pathophysiological consequences through different molecular mechanisms.

### Role of the transglutaminase activity in neuroinflammation

Neuroinflammation plays an important role in various chronic neurodegenerative diseases, characterized also by the pathological accumulation of specific protein aggregates. In particular, several of these proteins have been shown to be substrates of transglutaminases. Interestingly, it has recently been demonstrated that transglutaminase 2 (TG2) may also be involved in molecular mechanisms underlying inflammation. In the central nervous system, astrocytes and microglia are the cell types mainly involved in this inflammatory process. The transcription factor NF-kB is considered the main regulator of inflammation and it is activated by a variety of stimuli including calcium influx, oxidative stress and inflammatory cytokines. Recently, in addition to these stimuli, TG2 has been shown to activate NF-kB both via a canonical pathway and via a non-canonical pathway. On the other hand, NF-kB regulatory response elements are present also in the TG2 promoter. Under these conditions, the over-expression of TG2 results in the sustained activation of NF-kB. Several findings emphasize the possible role of the TG2/NF-kB activation pathway in neurodegenerative diseases, including Alzheimer’s disease, Parkinson’s disease, multiple sclerosis and amyotrophic lateral sclerosis. Together, these evidences suggest that TG2 could play a role in neuroinflammation and could contribute to the production of compounds that are potentially deleterious to neuronal cells.

### Transglutaminase inhibition as possible therapeutic approach in neurodegenerative diseases

In consideration to the fact that up to now there have been no long-term effective treatments for the human neurodegenerative diseases previously reported, then the possibility that selective TG inhibitors may be of clinical benefit has been seriously considered. In this respect, some encouraging results have been obtained with TG inhibitors in preliminary studies with different biological models of CAG-expansion diseases. For example, cystamine is a potent inhibitor of enzymes that require an unmodified cysteine at the active site. Inasmuch as TGs contain a crucial active-site cysteine, cystamine has the potential to inhibit these enzymes by a sulfide-disulfide interchange reaction. A sulfide-disulfide interchange reaction results in the formation of cysteamine and a cysteamine-cysteine mixed disulfide residue at the active site. Recent studies have shown that cysteamine decreases the number of protein inclusions in transfected cells expressing the atrophin (DRPLA) protein containing a pathologically-long polyglutamine domain. In other studies, cystamine administration to

### Table 2. List of polyglutamine (CAG-expansion) diseases.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Sites of neuropathology</th>
<th>CAG triplet number</th>
<th>Gene product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corea Major or HD</td>
<td>Striatum (medium spiny neurons) and cortex in late stage</td>
<td>6-35</td>
<td>36-121</td>
<td>Huntingtin (n,c)</td>
</tr>
<tr>
<td>SCA1</td>
<td>Cerebellar cortex (Purkinje cells), dentate nucleus and brain stem</td>
<td>6-39</td>
<td>40-81</td>
<td>Ataxin-1 (n,c)</td>
</tr>
<tr>
<td>SCA2</td>
<td>Cerebellum, pontine nuclei, substantia nigra</td>
<td>15-29</td>
<td>35-64</td>
<td>Ataxin-2 (c)</td>
</tr>
<tr>
<td>SCA3 or MJD</td>
<td>Substantia nigra, globus pallidus, pontine nucleus, cerebellar cortex</td>
<td>13-42</td>
<td>61-84</td>
<td>Ataxin-3 (c)</td>
</tr>
<tr>
<td>SCA6</td>
<td>Cerebellar and mild brainstem atrophy</td>
<td>4-18</td>
<td>21-30</td>
<td>Calcium channel Subunit (α 1A)(m)</td>
</tr>
<tr>
<td>SCA7</td>
<td>Photoreceptor and bipolar cells, cerebellar cortex, brainstem</td>
<td>7-17</td>
<td>37-130</td>
<td>Ataxin-7 (n)</td>
</tr>
<tr>
<td>SCA12</td>
<td>Cortical, cerebellar atrophy</td>
<td>7-32</td>
<td>41-78</td>
<td>Brain specific regulatory subunit of protein phosphatase PP2A (7)</td>
</tr>
<tr>
<td>SCA17</td>
<td>Gliosis and neuronal loss in the Purkinje cell layer</td>
<td>29-42</td>
<td>46-63</td>
<td>TATA-binding protein (TBP) (n)</td>
</tr>
<tr>
<td>SBMA or Kennedy</td>
<td>Motor neurons (anterior horn cells, bulbar neurons)</td>
<td>11-34</td>
<td>40-62</td>
<td>Androgen receptor (n,c)</td>
</tr>
<tr>
<td>Disease</td>
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<tr>
<td>DRPLA</td>
<td>Globus pallidus, dentato-rubral and subthalamic nucleus</td>
<td>7-35</td>
<td>49-88</td>
<td>Atrophin (n,c)</td>
</tr>
</tbody>
</table>

HD, Huntington’s Disease; SCA1, Spinocerebellar Ataxia Type 1; SCA2, Spinocerebellar Ataxia Type 2; SCA3, Spinocerebellar Ataxia Type 3; MJD, Machado-Joseph disease; SCMA, Spinocerebellar Ataxia Type 6; SCA7, Spinocerebellar Ataxia Type 7; SCA12, Spinocerebellar Ataxia Type 12; SCA17, Spinocerebellar Ataxia Type 17; SBMA, Spinobulbar Muscular Atrophy; DRPLA, Dentatorubral-pallidoluysian Atrophy.
HD-transgenic mice resulted in an increase in life expectancy and amelioration of neurological symptoms. Although all these scientific reports seem to support the hypothesis of a direct role of transglutaminase activity in the pathogenesis of the polyglutamine diseases, cystamine is also found to act in the HD-transgenic mice by mechanisms other than the inhibition of Tgs, such as the inhibition of caspases, suggesting that this compound can have an additive effect in the therapy of HD. Currently, cystamine is already in phase I studies in humans with HD, but several side effects, such as nausea, motor impairment and dosing schedule have been reported as reasons for non-adherence during phase II studies in human patients affected by cystinosis.

Another critical problem in the use of TG inhibitors in treating neurological diseases relates to the fact that, as previously reported, the human brain contains at least four Tgs, including TG1, 2, 3 and TG6, and a strong non-selective inhibitor of Tgs might also inhibit plasma Factor XIIa, causing a bleeding disorder. Therefore, from a number of standpoint it would seem that a selective inhibitor, which discriminates between Tgs, would be preferable to an indiscriminate TG inhibitor. In fact, although most of the TG activity in mouse brain, at least as assessed by an assay that measures the incorporation of radioactive putrescine (amine donor) into N,N-dimethyl casein (amine acceptor), seems to be due to TG2, no conclusive data have been obtained by TG2 gene knock-out experiments about the involvement of this TG in the development of the symptoms in HD-transgenic mice. Moreover, a recent scientific report showed that cystamine reduces aggregate formation in a mouse model of oculopharyngeal muscular dystrophy (OPMD), in which also the TG2 knockdown is capable of suppressing the aggregation and the toxicity of the mutant protein PABPN1, suggesting this compound as a possible therapeutic for OPMD.

Conclusions

In conclusion, numerous scientific reports have investigated aberrant TG activity both in cancer and in neurodegenerative diseases, but still today we are looking for experimental findings which could definitely confirm the direct involvement of Tgs in the pathogenetic mechanisms responsible for these diseases. However, as result of the putative role of specific TG isoforms, such as TG2, in some human diseases, there is a considerable interest in developing inhibitors of these enzymes. Among those currently available, cystamine is the most commonly experimentally used to inhibit TG2 activity. In addition to cystamine, several types of TG2 inhibitors have been developed up to now. Interestingly, some of these inhibitors have shown promising results in experimental diabetic models. Therefore, the use of these inhibitors of Tgs could be then useful also for other clinical approaches. To minimize the possible side effects, however, more selective inhibitors of the Tgs should be required in the future. Progress in this area of research could be achieved, if possible, also through pharmacogenetic approaches.

References


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