Amino Acids (2007) 33: 385–394
DOI 10.1007/s00726-007-0517-0
Printed in The Netherlands

Amino Acids

Tissue transglutaminase and the stress response

Review Article

R. Ientile[1], **D. Caccamo**[1], and **M. Griffin**[2]

[1] Department of Biochemical, Physiological and Nutritional Sciences, University of Messina, Messina, Italy
[2] School of Life and Health Sciences, Aston University, Birmingham, U.K.

Received November 14, 2006
Accepted February 1, 2007
Published online March 28, 2007;

Summary. The expression of the protein crosslinking enzyme tissue transglutaminase (TG2, tTG), the ubiquitous member of transglutaminase family, can be regulated by multiple factors. Although it has been suggested that TG2 can be involved in apoptotic cell death, high levels of enzyme have also been associated with cell survival in response to different stimuli. Furthermore, evidence indicates that increases in TG2 production cause enzyme translocation to cell membrane. Cell stress can also lead to TG2 accumulation on the cell surface and in the extracellular matrix resulting in changes in cell-matrix interactions.

Here, we discuss the underlying mechanisms of TG2 up-regulation induced by various stimuli including glutamate exposure, calcium influx, oxidative stress, UV, and inflammatory cytokines.

These findings agree with a postulated role for transglutaminases in molecular mechanisms involved in several diseases suggesting that cross-linking reactions could be a relevant part of the biochemical changes observed in pathological conditions.

Keywords: Transglutaminases – Tissue transglutaminase – Neurodegeneration – Oxidative stress – Inflammation – Extracellular matrix

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid; CD, celiac disease; ECM, extracellular matrix; FN, fibronectin; GYKI 52466, 1-(4′-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzo-diazepine; IFN-γ, interferon-γ; LPA, lysophosphatidic acid; TGF-beta, transforming growth factor-beta; MK-801, (+)-5-Methyl-10,11-dihydro-5*H*-dibenzo [*a, d*]-cyclohepten-5,10-imine hydrogen maleate; NF-κB, nuclear factor-kappa B; NMDA, N-methyl-D-aspartic acid; 3-NP, 3-nitropropionic acid; PLA2, phospholipase A2; ROS, reactive oxygen species; TG(s), transglutaminase(s); TG2, tissue transglutaminase

Introduction

Protein aggregates are readily detected in a variety of physiological and pathological conditions suggesting they play an important role in both normal conditions and disease states in living cells at the intra- and extra-cellular level. Much emphasis has been placed on the enzymatic activity displayed by transglutaminases (TGs), since their activity and expression is often associated with adaptive protein modifications following different stimuli including the stress response. Members of the TG family are found in both the intracellular and extracellular environment, and are encoded by nine different human genes. Six of the related gene products have been characterized as enzymes showing a calcium-dependent transamidating activity leading to protein modification either by intra- or inter-protein cross-linking or polyamine incorporation. Formation of these protein cross-links can lead to the production of protein aggregates with increased resistance to breakage and chemical and proteolytic hydrolysis (Griffin et al., 2002). Tissue transglutaminase (TG2, tTG) is the most ubiquitous member of this family, which in addition to its transamidating activity can bind and hydrolyse GTP implicating its involvement in intracellular signalling. The binding of either GTP or GDP negatively modulates the Ca^{2+}-dependent transamidating activity of the enzyme by obstructing access to the active site (Griffin et al., 2002). As a consequence, inside the cell, under normal conditions, Ca^{2+} activation of the enzyme is very tightly regulated. TG2 localizes mainly in the cytoplasm, yet recent reports also suggest its presence in the nucleus, mitochondria, at the cell surface and in the extracellular matrix (ECM) (Griffin et al., 2002; Piacentini et al., 2005).

TG2 expression is constitutive in many different cell types, and depending on the cell type can be regulated by several transcriptional activators, such as cytokines,

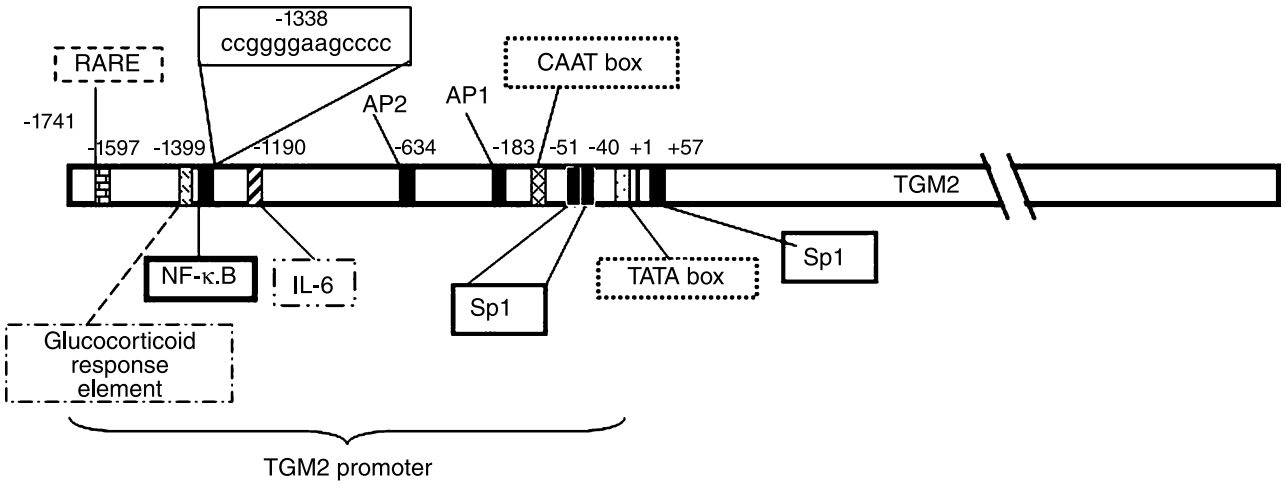

Fig. 1. Schematic representation of the proximal region of the human tissue transglutaminase gene (TGM2) promoter. The location of putative recognition motifs for known transcription factors, cytokines and steroid hormones was derived by published features of TGM2 promoter sequence (accession number: U13920 Z46905) except for that of NF-κB which was reported by Mirza et al. (1997). The transcription start site is numbered as *+1*. *RARE* Retinoic acid response element

retinoids, vitamin D and steroid hormones (Fig. 1). Given its multifunctional role, TG2 has been involved in a variety of events including the suppression of cell proliferation, differentiation, signal transduction and wound healing (Fèsus and Piacentini, 2002; Telci and Griffin, 2006).

Moreover, several observations suggest that TG2 could be involved in apoptosis whereby TG2-dependent cross-linking following increases in intracellular Ca^{2+} may be important in the stabilization of apoptotic cells by preventing loss of intracellular components (Fèsus and Szondy, 2005). Such a role has also been suggested for TG2 in the stabilization of necrotic cells following loss of Ca^{2+} homeostasis as a further mean of preventing cell leakage (Nicholas et al., 2003). Other results indicate that induced expression of TG2 by RA treatment (Boehm et al., 2002) or stable transfection with TG2 cDNA constructs (Tucholski and Johnson, 2002) does not produce any changes in the rate of spontaneous apoptosis in human neuroblastoma SH-SY5Y cells, confirming the tight regulation of the enzyme under normal Ca^{2+} homeostasis. Further work is required to elucidate the role of TG2 in the apoptotic process.

Several observations have been reported demonstrating that various stimuli or insults are able to induce TG2 activation following cell stress or injury. Further, many different stimuli, both internal and external, trigger endogenous production of reactive oxygen species (ROS) as a necessary part of the intracellular communication system that activates the cell stress response. ROS increase has been associated with different responses involving enzyme expression and post-translational changes that generally result in an adaptive response or loss of cell function. Here, we discuss the possibility that TG2 expression and subsequent transamidating activity could be part of the cell response evoked by stress conditions when cell homeostasis is disturbed. We have also elaborated on the regulatory mechanisms of TG2 gene expression in different stress models.

TGs and excitotoxic cell damage

In the nervous system, TG2 has been postulated to play a role in synaptic plasticity and release of neurotransmitters, long-term potentiation, and neuronal differentiation (Tucholski and Johnson, 2003; Tucholski et al., 2006). Additionally, several neurodegenerative conditions such as Alzheimer's disease and Huntington's disease have been associated with high levels of TG2 activity and protein expression (Gentile and Cooper, 2004). Many findings show that increases in Ca^{2+}-dependent TG reactions could be of pathological relevance. TGs may contribute to the formation of protein aggregates and to the biochemical changes occurring in cell damage and neuronal loss. The accumulation of insoluble oligomers and polymers of specific proteins initiate or contribute to neuronal dysfunction.

High glutamate concentrations in inter-synaptic spaces are a feature common to various neuro-pathological conditions and neurodegenerative diseases, such as Alzheimer's disease, Huntington's diseases, and amyotrophic lateral sclerosis.

Experiments, carried out with different agonists of glutamate receptors, have shown that stimulation of

N-methyl-D-aspartate (NMDA) receptors give onset to different calcium-dependent processes, mainly evoked by NMDA-receptor activation. Using primary cultures of cerebellar granule cells, we previously demonstrated an early increase in TG activity and TG2 expression after excitotoxic injury produced by a brief exposure to NMDA. These increases were strongly reduced by (+)-5-methyl-10,11-dihydro-5*H*-dibenzo [*a, d*]-cyclohepten-5,10-imine hydrogen maleate (MK-801), a non-competitive blocker of Ca^{2+} influx through NMDA receptor-gated ion channel (Ientile et al., 2002). In contrast, a pre-treatment with 1-(4′-aminophenyl)-4-methyl-7,8-methylenedioxy-5*H*-2,3-benzo-diazepine (GYKI 52466), a selective non-competitive antagonist of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid/kainic acid (AMPA/KA) receptors, was unable to significantly affect enzyme activity. This demonstrated that changes in TG expression and activity were a receptor-mediated event, specifically relying on the activation of the NMDA receptor subtype.

Further, in vitro observations have shown that astrocytes respond to toxic stimulus by different transduction mechanisms, including influx of Ca^{2+} as well as increased expression of immediate early genes. In this context, we previously demonstrated that increases in TG activity and TG2 expression were a function of cell differentiation in primary cultures of astrocytes treated with glutamate (100 μM) for 24 h. Notably, glutamate effects were significantly reduced when cell cultures were pre-incubated with GYKI 52466, an AMPA/KA receptor inhibitor, demonstrating a direct responsiveness of TG to glutamate receptor activation. We also observed a glutamate-induced active translocation of TG2 to the nuclear compartment in differentiated astrocytes (Campisi et al., 2003). This is relevant, considering a postulated role for TG2 in neurodegenerative diseases. In particular, nuclear inclusions in brains of subjects affected by Huntington's disease were associated with high levels of increased TG activity in different cell compartments. Indeed, cytosolic and intranuclear inclusions, which occur in neurodegeneration, are likely to play a role in the progression of the disease. Therefore, TG2 may contribute to the stabilization of protein aggregates, which, in turn, may lead to impairment of energy metabolism, resulting in cell death.

Although further experiments on glutamate-evoked effects in glial cells should be carried out, there is increasing evidence for cell damage in brain regions, executed by intracellular caspases which like TG2 also require Ca^{2+} for activity. In particular, astrocyte cell death, occurring during in vitro ischemia and AMPA receptor-mediated toxicity, has been associated with proteolytic activation of caspase-3 (Liu et al., 2002). We also demonstrated that glutamate-evoked increases in TG activity and TG2 expression in primary cultures of astrocytes paralleled the activation of the caspase pathway (Ientile et al., 2003). Caspase-3 activation like TG2 activation might therefore also be due to intracellular Ca^{2+} overload, triggered by glutamate receptor stimulation. Furthermore, it has been hypothesized that competitive primary amine substrates of TG are able to reduce cell damage associated with increases in aggregates formation. In particular, cell death was partially suppressed by cystamine and monodansylcadaverine (Igarashi et al., 1998). Indeed, under our experimental conditions, increases in both TG and caspase were reduced after incubation with cystamine prior to glutamate treatment, suggesting that changes in these enzymatic activities may be concomitant events subsequent to glutamate injury. Therefore, it can be suggested that an excess of glutamate leads to astrocyte death, an event which, if occurring in vivo, could impair brain functions, due to loss of the buffering capacity of astrocytes resulting in toxicity to neurons.

Neurodegeneration occurring in selectively vulnerable regions after global cerebral ischemia is morphologically indistinguishable from neuronal death caused by excitotoxicity, and, specifically, closely resembles cell death evoked by NMDA activation in the adult brain. Different experimental models have been used to study biochemical features of brain cell damage following ischemic insults. Numerous events, such as release of excitatory amino-acids, ROS production, and intracellular Ca^{2+} overload, are involved in the progression of ischemic neuronal loss, an active process caused by transient or permanent reduction of the cerebral blood flow. Interestingly, TG activity is reported to be associated with stress response in brain areas susceptible to ischemic damage (Paschen et al., 1990).

In a gerbil model of global cerebral ischemia, we evaluated changes in TG activity and expression during 48 h of reperfusion after a 3 min carotid occlusion. The time course of enzyme activity provided evidence for increased TG reactions in the early stages of ischemic injury and throughout the first 24 h. In particular, TG was found to be strongly activated in the ischemic hippocampus, while only minor modifications were observed in the cerebral cortex. Robust evidence was also provided for the presence of different TG isoenzymes in the ischemic brain regions. Indeed, a four-fold and two-fold increased expression of TG2 and TG1, respectively, were found in the hippocampus following 24 h of reperfusion. Interestingly, low mRNA levels were found for TG3, particularly in hippocampus of both ischemic and sham-operated gerbil

brains. These data agree with previous observations demonstrating that gene products of the TG gene cluster are differentially expressed in tissues (Grènard et al., 2001a). Notably, despite the differential expression and apparently distinct promoter organization, the expression patterns of TG2, TG5 and, to a more limited degree, TG3 overlap, suggesting a functional redundancy in the TG gene family. The close similarity of these gene products, and their overlapping functions, may explain the lower expression of TG3 vs TG2 observed both in normal and ischemic hippocampus, and the lack of TG5 in these same tissues.

Moreover, the observed region-specific changes in TG expression and activity in the brain were probably dependent on the different susceptibilities to ischemia of the examined brain areas. In fact, it is well known that the hippocampus is more severely damaged by ischemic insult, while brain cortex is only moderately affected. Further, the activation of Ca^{2+} channels is sharply increased in hippocampus during and after ischemic events (Lipton, 1999). However, it has been reported that the expression of TG, promoting cell survival or delayed cell death in different death models, is often altered in cell type-specific patterns (Fésus and Szondy, 2005).

TG and oxidative stress

Increasing evidence shows that oxidative stress among other processes is implied in the progression of neuronal death and that excitotoxicity and oxidative stress are interdependent phenomena. Oxidative stress occurs when cell defenses against ROS production fail. This leads to ROS accumulation that has a number of direct and indirect consequences on cell signalling pathways and may result in apoptosis or necrosis. Therefore, much attention has been paid to elucidate the potential role(s) played by TG2 in oxidative stress-induced cell death both under physiological and pathological conditions. This is particularly important given the multifunctional roles now ascribed to TG2 which include its possible role in the apoptosis (Piacentini et al., 2005), as a fail safe component in necrosis (Nicholas et al., 2003), and its recently documented role as an intracellular survival protein (Fésus and Szondy, 2005).

The first in situ observation that oxidative stress increases TG activity was made by Lesort et al. (2000), which showed that impairment of mitochondrial function following treatment with 3-nitropropionic acid (3-NP), an irreversible inhibitor of succinate dehydrogenase, significantly increased TG activity in neuroblastoma SH-SY5Y cells.

Further, exposure to 3-NP in combination with antioxidants was able to reduce the 3-NP-induced increase of in situ TG activity, demonstrating that oxidative stress is a contributing factor to the increase of TG activity. Strikingly, it has also been shown that TG overexpression sensitized the neuronal cell line SK-N-BE to apoptosis through constitutive mitochondrial hyperpolarization and increases in ROS production (Piacentini et al., 2002). These results could also be relevant in the pathogenesis of those diseases that are characterized by increased TG2 and apoptotic rate together with impaired mitochondrial function, e.g. in some neurodegenerative diseases.

The involvement of TGs in the pathogenesis of several chronic neurodegenerative diseases in which oxidative stress is a prominent feature, such as Alzheimer's disease and Huntington's disease, has been reported (Kim et al., 2002; Gentile and Cooper, 2004). In these pathological conditions, TGs appear to be responsible for the inappropriate formation and/or stabilisation of protein aggregates that may be cytotoxic. Indeed, protein misfolding dependent on oxidative effects may also make proteins better substrates for TG-catalyzed cross linking, leading to polymer formation, i.e. those of protein tau, as a result of the disease process.

Interestingly, in several neurodegenerative disorders an age-related sensitivity of astrocytes to oxidative stress has been observed, which makes neurons more susceptible to injury.

In this context, previous studies have shown that stimulation of cultured astrocytes with high glutamate concentrations results in oxidative stress, as indicated by the reduction of GSH levels and overproduction of intracellular ROS (Campisi et al., 2004). It has been reported that this altered redox status is consistent with a glutamate uptake-induced impairment of cystine/glutamate antiporter leading to GSH depletion. Notably, TG2 up-regulation was associated with the cell response to oxidative stress under our experimental conditions. The causative role of oxidative stress in TG2 increases was confirmed by both inhibition of ROS production and intracellular GSH depletion through pre-incubation with GSH ethyl ester or cysteamine-HCl. In fact, the recovery of redox status, achieved by these agents, was accompanied by a concomitant, dose-dependent, reduction of glutamate-induced TG2 up-regulation. In this regard, the greater effect observed in the presence of GSH ethyl ester could reside in its more powerful action, than cysteamine-HCl, in restoring ROS control levels (Campisi et al., 2004).

Activation of nuclear factor-kappa B (NF-κB), one of the most studied transcription factor families, has long

been known to be involved in oxidative stress in numerous cell types. Further, it seems to play a key role in redox status alterations triggered under different conditions in both neurons and astroglial cells (Blondeau et al., 2001). We previously demonstrated that glutamate exposure activated the NF-κB pathway in primary cultures of astrocytes, and that glutamate effects were significantly reduced by the pre-incubation with the antioxidants GSH ethyl-ester, cysteamine-HCl, genistein, and IRFI-016, a synthetic α-tocopherol analogue (Caccamo et al., 2005a, b). In the light of these observations, it was investigated whether glutamate-induced TG2 up-regulation in this experimental model could be mediated by NF-κB activation. Interestingly, competition experiments, carried out with an oligonucleotide probe containing the NF-κB consensus sequence present in the TG2 promoter and an excess of a probe containing the NF-κB motif present in the κ-light-chain promoter, demonstrated a preferential binding of TG2 specific NF-κB probe in the nuclear extracts of glutamate-treated astrocytes compared with untreated astrocytes (Caccamo et al., 2005c).

Further, the pre-incubation with the specific NF-κB inhibitor SN50 (Lin et al., 1995) (but not its inactive mutated analogue, SN50M), blocked NF-κB nuclear translocation and reduced glutamate-increased TG2 expression. These preliminary data suggest that the NF-κB activation, involved in the astrocyte response to glutamate-induced oxidative stress, may also be associated with the molecular pathways leading to glutamate-evoked TG2 up-regulation. However, it has been reported that LPS-increased TG activity in BV-2 microglia leads to NF-κB activation via an IKK-independent pathway. This alternative pathway implies a TG-mediated I-κBα polymerization and an ubiquitin/proteasome-driven degradation (Lee et al., 2004). Therefore, it can be hypothesized that the high levels of TG activity observed in this primary astrocyte experimental model could, in turn, sustain NF-κB activation as a central pathway in cell defense against redox state alterations triggered by glutamate. Since the neuroprotective role for NF-κB in brain oxidative stress induced by different stimuli is well documented (Blondeau et al., 2001), it is likely that TG2 also acts as a survival protein in this model of cell damage. This agrees with recent reports focusing on the role of TG2 in preventing cell death depending on the kind of stimuli and cell type (Fésus and Szondy, 2005).

Intracellular ROS have also been shown to be essential for the activation of in situ TG in response to lysophosphatidic acid (LPA) and transforming growth factor-beta (TGF-beta) in Swiss 3T3 fibroblasts. TG-mediated incorporation of 5-(biotinoamido)-pentylamine was strongly induced by both LPA and TGF-beta, and inhibited by cystamine and ROS scavengers. The role of intracellular ROS in the LPA- and TGF-beta-increased TG activity was also supported by the activation of in situ TG by exogenous H_2O_2 (Lee et al., 2003).

Interestingly, it has been suggested that intracellular ROS also mediate the activation of TG2 and stress fiber formation induced by arachidonic acid. Indeed, arachidonic acid elevated TG activity in a dose- and time-dependent manner in NIH3T3 cells, and ROS scavengers, N-(2-mercaptopropionyl)glycine and catalase were able to block the arachidonic acid effects (Yi et al., 2004).

Recently, it has been reported that oxidative stress or UV irradiation induced an aberrant in situ TG activity in human lens epithelial cells. Further, screening of a number of cell lines revealed that the level of TG activation was dependent on the cell type and also the environmental stress, suggesting that unrecognized cellular factor(s) may specifically regulate in situ enzyme activity. The activated TG2 catalyzed the in vitro formation of water-insoluble dimers or polymers of alphaB-crystallin, betaB(2)-crystallin, and vimentin in HLE-B3 cells, providing evidence that TG2 may play a role in cataractogenesis (Shin et al., 2004). A comparable observation was also seen in dermal fibroblasts after UVA irradiation resulting in the increased crosslinking of both intracellular and extracellular proteins (Gross et al., 2003).

TG and inflammation

A large body of evidence shows that increased TG activity and TG2 expression are found both in diseased tissue with inflammation and in cells with inflammatory stress. Although multiple physiological roles for TG2 have been demonstrated in various cell types, its role in the inflammatory process must be further clarified. A preliminary explanation is consistent with observations that inappropriate protein aggregates may be cytotoxic enough to trigger inflammation and/or cell damage. Recent studies indicate that inhibition of TGs will be a profitable new approach to the treatment of at least some types of inflammation. Indeed, the inflammatory process is currently effectively treated with glucocorticoids, which induces many proteins capable of inhibiting phospholipase A2 (PLA2). Activation of the secretory form of PLA2 (sPLA2) has been reported to be catalyzed by cell surface TG2. Therefore, blocking TG2 may ameliorate PLA2-mediated inflammatory diseases (Kim, 2006).

TG antibodies have been found in a number of inflammatory diseases, such as celiac disease, Type 1 diabetes, rheumatoid arthritis, lupus erythematosus, dermatitis herpetiformis and inclusion body myositis, suggesting the possibility that the inappropriate expression and/or presentation of TGs to T cells is also associated with the pathogenic progression, as well as with the generation of autoantibodies (Kim, 2006). Further, the presence of TG2 as autoantigen could also result from cell damage and leakage. In this regard, the high prevalence of anti-TG2 antibodies in the pathogenesis of Type 1 diabetes has been suggested following beta-cell destruction (Lampasona et al., 1999).

Celiac disease (CD) is one of the most common immune-mediated disorders, triggered by ingestion of wheat gluten and related cereal proteins. These induce an inflammatory response in the small intestine, resulting in villous atrophy, crypt hyperplasia, and lymphocytic infiltration (Green and Jabri, 2003). CD is also strongly associated with the presence of autoantibodies against gluten proteins and connective tissue components, the main target of which is TG2. The mechanisms by which gliadin and other prolaminic fractions of ''toxic'' proteins damage the intestinal mucosa of celiac patients are still unknown, but there is now evidence indicating that the damage is immunologically mediated. The activation of an adaptive immune response subsequently leads to production of high levels of pro-inflammatory cytokines, such us IL-1, IL-18 and chemokines, which are responsible for maintaining the inflammation and mucosal damage. Notably, TG2 is known to be induced by inflammatory cytokines, such as IL-1β in brain astrocytes (Monsonego et al., 1997), TNF-α in liver cells (Kuncio et al., 1998), and IFN-γ in intestinal cells (Kim et al., 2002).

Evidence also demonstrates that TNF-α and cytokines trigger a series of intracellular events that result in the activation of transcription factors, such as NF-κB and c-Jun (Chen and Goeddel, 2002), suggesting that TG2 can also be directly induced by NF-κB activation, since the TG2 promoter has an NF-κB binding motif (Mirza et al., 1997) (see Fig. 1). As alluded to earlier a TG-mediated polymerization of NF-κB inhibitory protein, I-κBα, results in the direct activation of NF-κB (Lee et al., 2004). Confirmation of this effect was obtained by the observation that TG2 inhibition reverses NF-κB activation. Interestingly, this coincides with the reversal of inflammation in conjunctivitis models by treatment with TG2 inhibitors (Lee et al., 2004).

Although the above observations suggest that TG2 is involved in the pathogenesis of celiac disease, further studies should be carried out to ascertain whether its role is direct or indirect. However, marker autoantibodies directed against either the endomysium of transitional epithelium (EMA) or TG2 are highly correlated and used in the diagnosis of CD.

Notably, TG2 generates autoantibodies in a variety of autoimmune disorders by cross-linking potential autoantigens and acting as a hapten. This hypothesis is supported by numerous reports showing the participation of TG substrates in autoimmune diseases (Kim et al., 2002). Anti-TG2 can be found in serum samples from patients with other inflammatory diseases, such as lupus erythematosus or rheumatoid arthritis (Marai et al., 2004; Song and Choi, 2004). Indeed, the possible presence of anti-TG2 in other inflammatory diseases should also be addressed.

Interestingly, despite TG2 being implicated as the major autoantigen of gluten sensitive diseases, another enzyme of the TG family was also shown to be effective as autoantigen in these pathologies. In a recent report, it was demonstrated that while sera from patients with CD react with both TG2 and the related TG3, antibodies present in patients having dermatitis herpetiformis showed a markedly higher avidity for epidermal TG3 (Sardy et al., 2002).

Chronic inflammation is also involved in sporadic inclusion body myositis, a progressive muscle disorder with an autoimmune origin (Kim, 2006). This disease is characterized by abnormal deposition of β-amyloid polymers in the muscle fiber, with subsequent muscle destruction, and has been associated with increased expression of both TG1 and TG2, which co-localize with the amyloid deposits. The up-regulation of both TGs seems to be stimulated by the excess production of IFN-γ and TNF-α by Th1 cells (Kim, 2006). Increased TG2 expression has also been reported in other idiopathic inflammatory miopathies, such as dermatomyositis and polymyositis (Choi et al., 2004).

TG, stress response and extracellular matrix

In addition to its intracellular functions related to cell trauma and stress, TG2 may also be translocated to the plasma membrane and subsequently deposited into the ECM via a non-classical secretory mechanism thought to be dependent on the active site conformation and an intact N-terminal beta-sandwich domain (Gaudry et al., 1999; Balklava et al., 2002).

Secretion of the enzyme to the cell surface and deposition into the ECM following cell trauma and stress is important in both modulating the inflammatory response via crosslinking of the secretory form of PLA2 (Kim, 2006) and in the remodeling and/or stabilization of the

several ECM proteins, such as fibronectin (FN) (Skill et al., 2004; Telci and Griffin, 2006). FN is particularly important since TG2 binds to this ECM protein with high affinity promoting wide ranging effects on cell-matrix interactions important in tissue remodeling and wound healing, including the regulation of cell adhesion and migration, matrix assembly, and adhesion-dependent signaling (Akimov et al., 2000; Verderio et al., 2004; Telci and Griffin, 2006).

TG2 can enhance the cell adhesion and migration of different cell types by the direct modification of ECM proteins through their cross-linking (Chau et al., 2005). In addition, recent reports describe TG2 acting as a novel cell adhesion protein (Akimov et al., 2000; Takahashi et al., 2000) by mechanisms which are independent of its transamidating activity. The importance of TG2 as a matrix associated wound response enzyme is well reported (Telci and Griffin, 2006). Under these conditions a matrix rich in both TG2 and FN (TG-FN) may be found in which increased deposition of TG2, either by increased secretion from surrounding cells or via disruption of incoming red blood cells, occurs at injury sites where TG2 binds to FN with high affinity. Using either a TG-FN matrix or a cell-mediated TG2 rich matrix we have shown that TG-FN could restore loss of cell adhesion and promote cell survival in a number of cell types following the inhibition of the classical FN RGD-dependent cell adhesion pathway mediated by $\alpha_5\beta_1$ integrin receptors (Verderio et al., 2003).

In this study, we demonstrated that the digestion of cell surface heparan sulfate chains and the treatment with a protein kinase Cα inhibitor significantly reduced the cell adhesion to TG-FN after RGD inhibition, suggesting the involvement of protein kinase Cα in a downstream heparan sulphate proteoglycan receptor-mediated cell adhesion process (Verderio et al., 2003).

Such a process would be important during tissue injury and during matrix remodeling where disruption of FN and other matrix proteins leads to generation of soluble RGD containing peptides which can compete for matrix cell binding sites (Midwood et al., 2004; Telci and Griffin, 2006). RGD-independent cell adhesion to the TG-FN complex did not require transamidating activity but induced the formation of focal adhesion contacts, the assembly of associated actin stress fibers, and FAK phosphorylation.

In addition to the ability of TG2 to influence cell adhesion and migration the enzyme once secreted in significant quantities, generally following cell trauma or stress, into the ECM can also affect matrix deposition and turnover (Gross et al., 2003; Skill et al., 2004). A number of ECM proteins such as fibronectin, fibrinogen, osteonectin, osteopontin, collagens, vitronectin, collagen-tailed acetylcholinesterase, elafin, plasminogen activator inhibitor 2, and the large latent TGF-beta1 binding protein may act as substrates for TG2 (Verderio et al., 1999; Aeschlimann and Thomazy, 2000). Other cell surface/extracellular substrates include the secretory form of PLA2 referred to above which can lead to enhancement of the inflammatory response. However, the normal wound healing response of TG2 may also become pathological if the cell trauma/tissue insult is maintained leading to increased inflammation, tissue fibrosis and scarring (Verderio et al., 2004).

The first demonstration of TG involvement in fibrosis was given in 1979, by Griffin and co-workers, which observed changes in TG activity in an experimental model of pulmonary fibrosis induced by paraquat (Griffin et al., 1979). Subsequent to this the involvement of TG2 in kidney scarring is now well documented both in animal models (Johnson et al., 1997) and in human biopsy material (Johnson et al., 2003). Cell stress induced in proximal tubular epithelial cells by exposure to elevated glucose is one example of a stress-related response giving rise to increased TG2 secretion. An event which is likely to contribute to the pathological events associated with the progression of interstitial fibrosis and scarring in diabetic nephropathy (Skill et al., 2004).

Additionally, TG2 has also been found to be involved in ECM accumulation and deposition in severe chronic inflammatory states, including liver diseases (cirrhosis and fibrosis, alcoholic hepatopathy and type C hepatitis) (Mirza et al., 1997; Grènard et al., 2001b). Overexpression of TG2 in the heart caused interstitial fibrosis in transgenic mice but showed no evidence for the involvement of the GTPase function of the enzyme in adrenergic receptor signalling (Small et al., 1999). This points to a central role for TG2 in the regulation of fibroblast activities. Importantly, a major function for TG2 in tissue repair is also suggested by the recently reported abnormal wound healing response and reduced macrophage activity in TG2 knockout mice (Szondy et al., 2003).

Loss of homeostasis in ECM turnover is also integral to the pathology of the development of the atherosclerotic plaque. Accumulating evidence suggests that the attenuation of ECM and the instability of atherosclerotic plaque might lead to the erosion of the fibrous cap and plaque rupture, and that these pathological changes might initiate acute coronary syndrome. TG2 and elafin – an ECM substrate protein for TG2 – act as stabilizing factors, and might play a crucial role in the pathogenesis of acute coronary syndrome, since their expression increased with the

progression of coronary atherosclerosis. Further, in vitro experiments with cultured smooth muscle cells suggested that TG2 up-regulation might be mediated by TNF-α (Sumi et al., 2002).

Very recently a further function has been indicated for TG2 in the crosslinking of small heat-shock proteins (sHsps) which is enhanced by stress under pathological conditions; thus providing further evidence for a regulatory function of TG2 activity in the stress response (Boros et al., 2006).

Conclusions

A number of cell stress conditions, such as perturbed calcium homeostasis, alterations of redox status, increases in cytokines and mediators of inflammation, can interfere with gene expression leading to changes in the post-translational modification of proteins. These responses are much more distinct and specific to the individual agents than the corresponding responses. NF-κB is activated as part of the cell response and is thought to orchestrate a cell survival pathway. In this respect several findings suggest that NF-κB and TG2 interplay.

Indeed, TG2 can be induced directly by NF-κB activation, because the TG2 promoter possess a NF-κB binding motif. TG2 also induces NF-κB activation via two different pathways, an IKK-independent pathway and an IKK-dependent pathway (Mirza et al., 1997; Lee et al., 2004).

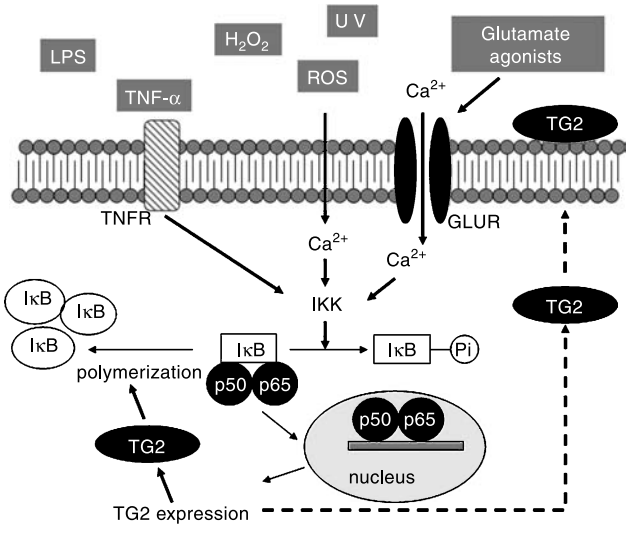

Fig. 2. Proposed mechanisms for TG2 induction in response to different stress conditions. In various cell lines NF-κB activation may be associated to increased TG2 expression; further, in response to different stimuli TG2 activity triggers NF-κB activation through I-kappaB-alpha polymerization. *TNFR* Tumor necrosis factor-a receptor; *GLUR* glutamate receptor, *IKK* IκBα kinase

However, it appears that the two pathways are reciprocally linked: NF-κB-induced TG2 expression in turn contributes to activation of NF-κB which results by I-κBα polymerization (see Fig. 2). Considering the relevance of TGs in inflammatory and fibrotic diseases, autoimmune conditions and neurodegenerative pathologies, safe, stable, and specific TG inhibitors may be effective agents in diseases associated with NF-κB activation improving the overall cell and tissue response to stress conditions.

Acknowledgement

We would like to thank Dr. Monica Currò for technical collaboration.

References

Aeschlimann D, Thomazy V (2000) Protein crosslinking in assembly and remodelling of extracellular matrices: the role of transglutaminases. Connect Tissue Res 41: 1–27

Akimov SS, Krylov D, Fleischman LF, Belkin AM (2000) Tissue transglutaminase is an integrin-binding adhesion coreceptor for fibronectin. J Cell Biol 148: 825–838

Balklava Z, Verderio E, Collighan R, Gross S, Adams J, Griffin M (2002) Analysis of tissue transglutaminase function in the migration of Swiss 3T3 fibroblasts: the active-state conformation of the enzyme does not affect cell motility but is important for its secretion. J Biol Chem 277: 16567–16575

Blondeau N, Widmann C, Lazdunski M, Heurteaux C (2001) Activation of nuclear factor-κB is a key event in brain tolerance. J Neurosci 21: 4668–4677

Boehm JE, Singh U, Combs C, Antonyak MA, Cerione RA (2002) Tissue transglutaminase protects against apoptosis by modifying the tumor suppressor protein p110 Rb. J Biol Chem 277: 20127–20130

Boros S, Ahrman E, Wunderink L, Kamps B, de Jong WW, Boelens WC, Emanuelsson CS (2006) Site-specific transamidation and deamidation of the small heat-shock protein Hsp20 by tissue transglutaminase. Proteins 62: 1044–1052

Caccamo D, Campisi A, Currò M, Aguennouz M, Li Volti G, Avola R, Ientile R (2005c) Nuclear factor-kappab activation is associated with glutamate-evoked tissue transglutaminase up-regulation in primary astrocyte cultures. J Neurosci Res 82: 858–865

Caccamo D, Campisi A, Currò M, Bramanti V, Tringali M, Li Volti G, Vanella A, Ientile R (2005b) Antioxidant treatment inhibited glutamate-evoked NF-kappaB activation in primary astroglial cell cultures. Neurotoxicology 26: 915–921

Caccamo D, Campisi A, Marini H, Adamo EB, Li Volti G, Squadrito F, Ientile R (2005a) Glutamate promotes NF-kappaB pathway in primary astrocytes: protective effects of IRFI 016, a synthetic vitamin E analogue. Exp Neurol 193: 377–383

Campisi A, Caccamo D, Li Volti G, Currò M, Parisi G, Avola R, Vanella A, Ientile R (2004) Glutamate-evoked redox state alterations are involved in tissue transglutaminase up-regulation in primary astrocyte cultures. FEBS Lett 578: 80–84

Campisi A, Caccamo D, Raciti G, Cannavò G, Macaione V, Currò M, Macaione S, Vanella A, Ientile R (2003) Glutamate-induced increases in transglutaminase activity in primary cultures of astroglial cells. Brain Res 978: 24–30

Chau DY, Collighan RJ, Verderio EA, Addy VL, Griffin M (2005) The cellular response to transglutaminase-cross-linked collagen. Biomaterials 26: 6518–6529

Chen G, Goeddel DV (2002) TNF-R1 signaling: a beautiful pathway. Science 296: 1634–1635

Choi YC, Kim TS, Kim SY (2004) Increase in transglutaminase 2 in idiopathic inflammatory myopathies. Eur Neurol 51: 10–14

Fèsus L, Piacentini M (2002) Transglutaminase 2: an enigmatic enzyme with diverse functions. Trends Biochem Sci 27: 534–539

Fèsus L, Szondy Z (2005) Transglutaminase 2 in the balance of cell death and survival. FEBS Lett 579: 3297–3302

Gaudry CA, Verderio E, Jones RA, Smith C, Griffin M (1999) Tissue transglutaminase is an important player at the surface of human endothelial cells: evidence for its externalization and its colocalization with the beta(1) integrin. Exp Cell Res 252: 104–113

Gentile V, Cooper AJ (2004) Transglutaminases – possible drug targets in human diseases. Curr Drug Targets CNS Neurol Disord 3: 99–104

Green PH, Jabri B (2003) Coeliac disease. Lancet 362: 383–391

Grènard P, Bates MK, Aeschlimann D (2001a) Evolution of transglutaminase genes: identification of a transglutaminase gene cluster on human chromosome 15q15. J Biol Chem 276: 33066–33078

Grènard P, Bresson-Hadni S, El Alaoui S, Chevallier M, Vuitton DA, Ricard-Blum S (2001b) Transglutaminase-mediated cross-linking is involved in the stabilization of extracellular matrix in human liver fibrosis. J Hepatol 35: 367–375

Griffin M, Casadio R, Bergamini CM (2002) Transglutaminases: nature's biological glues. Biochem J 368: 377–396

Griffin M, Smith LL, Wynne J (1979) Changes in transglutaminase activity in an experimental model of pulmonary fibrosis induced by paraquat. Br J Exp Pathol 60: 653–661

Gross SR, Balklava Z, Griffin M (2003) Importance of tissue transglutaminase in repair of extracellular matrices and cell death of dermal fibroblasts after exposure to a solarium ultraviolet A source. J Invest Dermatol 121: 412–423

Ientile R, Caccamo D, Macaione V, Torre V, Macaione S (2002) NMDA-evoked exctotoxicity increases tissue transglutaminase in cerebellar granule cells. Neuroscience 115: 723–729

Ientile R, Campisi A, Raciti G, Caccamo D, Currò M, Cannavò G, Li Volti G, Macaione S, Vanella A (2003) Cystamine inhibits transglutaminase and caspase-3 cleavage in glutamate-exposed astroglial cells. J Neurosci Res 74: 52–59

Igarashi S, Koide R, Shimohata T, Yamada M, Hayashi Y, Takano H, Date H, Oyake M, Sato T, Sato A, Egawa S, Ikeuchi T, Tanaka H, Nakano R, Tanaka K, Hozumi I, Inuzuka T, Takahashi H, Tsuji S (1998) Suppression of aggregate formation and apoptosis by transglutaminase inhibitors in cells expressing truncated DRPLA protein with an expanded polyglutamine stretch. Nat Genet 18: 111–117

Johnson TS, El-Koraie AF, Skill NJ, Baddour NM, El Nahas AM, Njloma M, Adam AG, Griffin M (2003) Tissue transglutaminase and the progression of human renal scarring. J Am Soc Nephrol 14: 2052–2062

Johnson TS, Griffin M, Thomas GL, Skill J, Cox A, Yang B, Nicholas B, Birckbichler PJ, Muchaneta-Jones RA, Nicholas B, Mian S, Davies PJ, Griffin M (1997) Reduced expression of tissue transglutaminase in a human endothelial cell line leads to changes in cell spreading, cell adhesion and reduced polymerisation of fibronectin. J Cell Sci 110: 2461–2472

Kim SY (2006) Transglutaminase 2 in inflammation. Front Biosci 11: 3026–3035

Kim SY, Jeitner TM, Steinert PM (2002) Transglutaminases in disease. Neurochem Int 40: 85–103

Kuncio GS, Tsyganskaya M, Zhu J, Liu SL, Nagy L, Thomazy V, Davies PJ, Zern MA (1998) TNF-alpha modulates expression of the tissue transglutaminase gene in liver cells. Am J Physiol 274: G240–G245

Lampasona V, Bonfanti R, Bazzigaluppi E, Venerando A, Chiumello G, Bosi E, Bonifacio E (1999) Antibodies to tissue transglutaminase C in type I diabetes. Diabetologia 42: 1195–1198

Lee J, Kim YS, Choi DH, Bang MS, Han TR, Joh TH, Kim SY (2004) Transglutaminase 2 induces nuclear factor-kappaB activation via a novel pathway in BV-2 microglia. J Biol Chem 279: 53725–53735

Lee ZW, Kwon SM, Kim SW, Yi SJ, Kim YM, Ha KS (2003) Activation of in situ tissue transglutaminase by intracellular reactive oxygen species. Biochem Biophys Res Commun 305: 633–640

Lesort M, Tucholski J, Zhang J, Johnson GV (2000) Impaired mitochondrial function results in increased tissue transglutaminase activity in situ. J Neurochem 75: 1951–1961

Lin YZ, Yao SY, Veach RA, Torgerson TR, Hawiger J (1995) Inhibition of nuclear translocation of transcription factor NF-κB by a synthetic peptide containing a cell membrane-permeable motif and nuclear localization sequence. J Biol Chem 270: 14255–14258

Lipton P (1999) Ischemic cell death in brain neurons. Physiol Rev 79: 1431–1536

Liu HN, Giasson BI, Mushynski WE, Almazan G (2002) AMPA receptor-mediated toxicity in oligodendrocyte progenitors involves free radical generation and activation of JNK, calpain and caspase 3. J Neurochem 82: 398–409

Marai I, Shoenfeld Y, Bizzaro N, Villalta D, Doria A, Tonutti E, Tozzoli R (2004) IgA and IgG tissue transglutaminase antibodies in systemic lupus erythematosus. Lupus 13: 241–244

Midwood KS, Williams LV, Schwarzbauer JE (2004) Tissue repair and the dynamics of the extracellular matrix. Int J Biochem Cell Biol 36: 1031–1037

Mirza A, Liu SL, Frizell E, Zhu J, Maddukuri S, Martinez J, Davies P, Schwarting R, Norton P, Zern MA (1997) A role for tissue transglutaminase in hepatic injury and fibrogenesis, and its regulation by NF-kappaB. Am J Physiol 272: G281–G288

Monsonego A, Shani Y, Friedmann I, Paas Y, Eizenberg O, 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

Nicholas B, Smethurst P, Verderio E, Jones R, Griffin M (2003) Cross-linking of cellular proteins by tissue transglutaminase during necrotic cell death: a mechanism for maintaining tissue integrity. Biochem J 371: 413–422

Paschen W, Rohn G, Schmidt-Kastner R (1990) Transglutaminase activity in reversible cerebral ischemia in the rat. Neurosci Lett 110: 232–236

Piacentini M, Amendola A, Ciccosanti F, Falasca L, Farrace MG, Mastroberardino PG, Nardacci R, Oliverio S, Piredda L, Rodolfo C, Autuori F (2005) Type 2 transglutaminase and cell death. Prog Exp Tumor Res 38: 58–74

Piacentini M, Farrace MG, Piredda L, Matarrese P, Ciccosanti F, Falasca L, Rodolfo C, Giammarioli AM, Verderio E, Griffin M, Malorni W (2002) Transglutaminase overexpression sensitizes neuronal cell lines to apoptosis by increasing mitochondrial membrane potential and cellular oxidative stress. J Neurochem 81: 1061–1072

Sardy M, Karpati S, Merkl B, Paulsson M, Smyth N (2002) Epidermal transglutaminase (TG 3) is the autoantigen of dermatitis herpetiformis. J Exp Med 195: 747–757

Shin DM, Jeon JH, Kim CW, Cho SY, Kwon JC, Lee HJ, Choi KH, Park SC, Kim IG (2004) Cell type-specific activation of intracellular transglutaminase 2 by oxidative stress or ultraviolet irradiation: implications of transglutaminase 2 in age-related cataractogenesis. J Biol Chem 279: 15032–15039

Skill NJ, Johnson TS, Coutts IG, Saint RE, Fisher M, Huang L, El Nahas AM, Collighan RJ, Griffin M (2004) Inhibition of transglutaminase activity reduces extracellular matrix accumulation induced by high glucose levels in proximal tubular epithelial cells. J Biol Chem 279: 47754–47762

Small K, Feng JF, Lorenz J, Donnelly ET, Yu A, Im MJ, Dorn GW, Liggett SB (1999) Cardiac specific overexpression of TG2 (Gh) results in a unique hypertrophy phenotype independent of PLC activation. J Biol Chem 274: 21291–21296

Song KS, Choi JR (2004) Tissue transglutaminase autoantibodies in patients with IgM rheumatoid factors. Yonsei Med J 45: 960–962

Sumi Y, Inoue N, Azumi H, Seno T, Okuda M, Hirata K, Kawashima S, Hayashi Y, Itoh H, Yokoyama M (2002) Expression of tissue transglutaminase and elafin in human coronary artery. Implication for plaque instability. Atherosclerosis 160: 31–39

Szondy Z, Sarang Z, Molnar P, Nemeth T, Piacentini M, Mastroberardino PG, Falasca L, Aeschlimann D, Kovacs J, Kiss I, Szegezdi E, Lakos G, Rajnavolgyi E, Birckbichler PJ, Melino G, Fesus L (2003) TG2-/- mice reveal a phagocytosis-associated crosstalk between macrophages and apoptotic cells. Proc Natl Acad Sci USA 100: 7812–7817

Takahashi H, Isobe T, Horibe S, Takagi J, Yokosaki Y, Sheppard D, Saito Y (2000) Tissue transglutaminase, coagulation factor XIII, and the pro-polypeptide of von Willebrand factor are all ligands for the integrins alpha 9beta 1 and alpha 4beta 1. J Biol Chem 275: 23589–23595

Telci D, Griffin M (2006) Tissue transglutaminase (TG2)-a wound response enzyme. Front Biosci 11: 867–882

Tucholski J, Johnson GV (2003) Tissue transglutaminase directly regulates adenylyl cyclase resulting in enhanced cAMP-response element-binding protein (CREB) activation. J Biol Chem 278: 26838–26843

Tucholski J, Johnson GVW (2002) Tissue transglutaminase differentially modulates apoptosis in a stimuli-dependent manner. J Neurochem 81: 780–791

Tucholski J, Roth KA, Johnson GVW (2006) Tissue transglutaminase overexpression in the brain potentiates calcium-induced hippocampal damage. J Neurochem 97: 582–584

Verderio E, Gaudry C, Gross S, Smith C, Downes S, Griffin M (1999) Regulation of cell surface tissue transglutaminase: effects on matrix storage of latent transforming growth factor-beta binding protein-one. J Histochem Cytochem 47: 1417–1432

Verderio EA, Johnson T, Griffin M (2004) Tissue transglutaminase in normal and abnormal wound healing: review article. Amino Acids 26: 387–404

Verderio EAM, Telci D, Okoye A, Melino G, Griffin M (2003) A novel RGD-independent cell adhesion pathway mediated by fibronectin-bound tissue transglutaminase rescues cells from anoikis. J Biol Chem 278: 42604–42614

Yi SJ, Choi HJ, Yoo JO, Yuk JS, Jung HI, Lee SH, Han JA, Kim YM, Ha KS (2004) Arachidonic acid activates tissue transglutaminase and stress fiber formation via intracellular reactive oxygen species. Biochem Biophys Res Commun 325: 819–826

Authors' address: Dr. Riccardo Ientile, Department of Biochemical, Physiological and Nutritional Sciences, University of Messina, Via Consolare Valeria, Policlinico Universitario, I-98125 Messina, Italy,
Fax: +39-90-221 3898, E-mail: ientile@unime.it