Nuclear Translocation of Tissue Type Transglutaminase during Sphingosine-Induced Cell Death: A Novel Aspect of the Enzyme with DNA Hydrolytic Activity

Yutaka Takeuchi[a], Hiroshi Ohashi[a], Paul J. Birckbichler[b] and Takashi Ikejima[a,*]

[a] Biomedical Research Laboratories, Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd., 3 Okubo, Tsukuba, Ibaraki 300-26, Japan
[b] Dept. of Urology, University of Oklahoma Health Sciences Center, Oklahoma City, Ok 73104, USA

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Tissue type (type 2) transglutaminase (TGase, EC 2.3.2.13) has been implicated in various cellular processes including cell death. In order to better understand the role of this enzyme in cell death, human melanocytic A375-S2 cells were treated with sphingosine, a cell-signaling mediator. During the rapid onset of cytotoxicity caused by this lipidic agent, tissue TGase was translocated from the cytoplasm to the cell nuclei. This observation was further remarked in relevance to its previously undescribed activity for DNA degradation. The DNA hydrolytic activity associated with tissue TGase was dependent on Mg^{2+} in contrast to the Ca^{2+} requirement for the classical cross-linking acrivity of TGase, and was inhibited by Zn^{2+}. Based on the results shown here, we propose a novel aspect of tissue TGase in cell death.

Introduction

Transglutaminase (TGase; R-glutaminyl-peptide:amine-γ-glutamyltransferase, EC 2.3.2.13) is an enzyme that catalyzes, in a Ca^{2+}-dependent manner, an acyl-transfer reaction between peptidyl glutamine residues and primary amines including proteinaceous ε-amino group of lysine residues (Folk and Finlayson, 1977; Lorand and Conrad, 1984; Chung, 1975; Greenberg *et al.*, 1991; Folk, 1980). Distinct isoforms of this enzyme family are known in various tissues and body fluids of mammals as well as plants and bacteria (Folk and Finlayson, 1977; Lorand and Conrad, 1984; Chung, 1975; Greenberg *et al.*, 1991; Folk, 1980; Aeschlimann *et al.*, 1994; Margosiak *et al.*, 1990; Ando *et al.*, 1989). Tissue type (type 2) TGase comprises a major subfamily of the enzyme. It is distributed in the cytoplasm of cells and has long been proposed to participate in various biological processes such as growth and differentiation of cells (Birckbichler *et al.*, 1981; Murtaugh *et al.*, 1983; Knight *et al.*, 1991), and more recently in cell death (Piacentini *et al.*, 1991; Piacentini and Autuori, 1994; Amendola *et al.*, 1996; Zhang *et al.*, 1995; Szondy *et al.*, 1997). Recent studies revealed further interesting aspects of physiological significance of tissue TGase. Firstly, the enzyme activity was pivotal in the ability of lesioned fish optic nerve to cross-link interleukin-2 molecules into a dimerized form, reducing the neurotoxicity of ambient oligodendrocytes and underlying regeneration of injured optic neurons (Eitan and Schwartz, 1993; Eitan *et al.*, 1994). Secondly, in addition to its conventional cross-linking activity, tissue TGase is a nucleotide binding protein with GTPase and ATPase activity (Achyuthan and Greenberg, 1987; Lee *et al.*, 1989; Takeuchi *et al.*, 1992; Lee *et al.*, 1993; Monsonego *et al.*, 1997; Lai *et al.*, 1996; Singh and Cerione, 1996; Takeuchi *et al.*, 1994). The demonstration of a novel function as a G protein in α_1-adrenoceptor-coupled cell signaling system (Nakaoka *et al.*, 1994; Chen *et al.*, 1996; Feng *et al.*, 1996) has evoked wider interest in the physiological relevance of the tissue TGase.

In this report, we expand the multifunctional activities of tissue TGase to include a possible role

Reprint requests to Dr. Y. Takeuchi.
Biomedical Research Laboratories, Banyu Tsukuba Research Institute, 3 Okubo, Technopark Oho, Tsukuba, Ibaraki 300–26, Japan.
Fax: 298-77-2027; E-mail: takeucyt@banyu.co.jp.

* Present address: Research Center of New Drug, Changchun College of Traditional Chinese Medicine, 20 Gong Nong Main Road, Changchun, Jilin Province 130021, People's Republic of China.

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in cell death. During sphingosine-triggered death of human melanocytic A375-S2 cells, we demonstrate a loss of tissue TGase from the cytoplasm and a concomitant translocation of the enzyme to the cell nuclei. We also propose a biological relevance to this translocation of the enzyme by showing that it possesses a DNA hydrolytic activity that is Mg^{2+}-dependent, but not Ca^{2+}-dependent, and that is inhibited by Zn^{2+}.

Materials and Methods

Cell culture and chemicals

A375-S2 cells (human melanoma cell line, ATCC CRL-1872, Nakai *et al.*, 1988) were maintained in fetal calf serum (10%)-containing RPMI 1640 medium, which was originally proposed for lymphocyte culture (Moore *et al.*, 1967). D-sphingosine and DL-erythro-dihydrosphingosine (DL-sphinganine) were the products of Sigma. These lipids were first dissolved in methanol, diluted with Hepes buffer containing bovine serum albumin (fatty acid-free grade, Sigma) and then with H_2O, resulting in a 2 mM solution in 25 mM Hepes (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4) containing 2 mg/ml bovine albumin and 10% methanol (a similar protocol was reported by Hauser *et al.*, 1994). Guinea pig liver TGase was purchased from Sigma and Takara (Kusatsu, Japan). Although these commercial preparations were electrophoretically confirmed for purity, we further applied them to GTP-agarose (Sigma) column chromatography prior to use (Lee *et al.*, 1989; Takeuchi *et al.*, 1994).

Analysis of cell death

Following the overnight incubation ($3.0x10^3$ cells per well on a 96-well plate, $5.0x10^5$ cells per 25 cm^2 flask, and $4.6x10^6$ cells per 225 cm^2 flask), cells were treated with vehicle buffer or either sphingosine or sphinganine (final concentration of 40 μM) that was preheated at 50 °C. Two minutes later (a significant portion of cell population already showed their morphological abnormality at this moment, see Fig. 1), cells were scraped from 225 cm^2 flasks, pelleted by a low speed centrifugation and washed twice with 3 ml phosphate-buffered saline at 4 °C. This step was performed in 4 min. Cell nuclei were separated from 1% NP-40 lysate and were pelleted as reported (Bates *et al.*, 1994). The post-nuclear cell lysate was concentrated to 300-400 μl by Centricon 30 (Amicon, MA) with 40 mM Tris (hydroxymethyl) aminomethane-HCl (pH 7.5) containing 150 mM sodium chloride, 0.5 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin and 1 mM dithiothreitol. The pelleted cell nuclei were briefly (4 sec x 3) sonicated in 300 μl of the same buffer (Takaku *et al.*, 1995). Both lysate and nuclear fractions were analyzed on sodium dodecylsulfate-polyacrylamide gel electrophoresis (8% acrylamide) followed by immunoblotting with a monoclonal antibody CUB74 (Birckbichler *et al.*, 1985). Tissue TGase was detected by ECL (enhanced chemiluminescence) method (Takaku *et al.*, 1995; Ohashi *et al.*, 1995), using a kit (Amersham). Cell death was estimated by assaying lactate dehydrogenase activity (Korzeniewski and Callewaert, 1983; Decker and Lohmann-Matthes, 1988) retained in the surviving cells on a 96-well plate, using a LDH-Cytotoxicity testing kit (Wako Pure Chemicals, Osaka). Cells were also monitored for their morphology under a light microscope.

DNA hydrolytic assay

pGEM-3Z plasmid (Promega, 0.8 μg) and purified guinea pig TGase (0.14 μg) were incubated at 37 °C for 1 h in 10 μl of 25 mM Tris-acetate buffer (pH 7.6) containing 10 mM magnesium chloride, 0.1 M potassium acetate, 0.5 mM dithiothreitol, 50 μM EDTA and 10 μg/ml bovine serum albumin. The mixture was then analyzed on 1% agarose electrophoresis. Effect of divalent cations (2 mM $CaCl_2$ and 2 mM $ZnCl_2$) was also tested.

Other assays

The TGase activity retained in the post-nuclear cell lysate was determined as incorporation of [^{14}C] putrescine into *N,N*- dimethylcasein (Ohashi *et al.*, 1995; Lorand *et al.*, 1972) after a 2 h reaction to assure detectable activity. The activity released into the culture medium was also determined after concentrated through a GTP-agarose column (Takeuchi *et al.*, 1994). Proteins were determined by the Bradford method (Bradford, 1976).

Results and Discussion

Severe toxicity of sphingolipids to A375-S2 cell line

Sphingosine and sphinganine function as lipidic mediators in the sphingomyelin signaling cycle, exerting multiple cellular effects on proliferation, survival and death processes (Kolesnick and Golde, 1994; Spiegel and Merrill, 1996; Hannun, 1996). A375-S2 cells started their death schedule by a sphingosine-treatment. After a 2 min exposure to sphingosine, morphological changes of the cells were already evident with the characteristic appearance of clusters of round-shaped cells (Fig. 1C). At 5 min or later, the damage to the cells was more prominent as featured by rough indentations and blebbing at the cell surface (Fig. 1D). Within 20 min, most cells became detached from the flask, aggrgated, and were floating in the culture medium (Fig. 1E). Sphinganine exerted a similar, though slightly milder, effect on the cells (data not shown). To the contrary, untreated control cells and vehicle (bovine albumin/ethanol)-treated mock cells (see Materials and Methods), maintained their normal appearance throughout this time scale (Fig. 1A, B and F). Concurrently, a rapid reduction in the lactate dehydrogenase activity was observed in the sphingosine- and sphinganine-treated cells (Table I), confirming a severe toxicity of such sphingolipids to A375-S2 cells. Concentration of these lipids lower than 20 μM produced only a transient change in cell morphology (data not shown).

Intracellular dynamics of tissue type TGase

We next examined the intracellular redistribution of tissue TGase provoked by a sphingosine treatment of A375-S2 cells. In a preliminary experiment, bovine albumin, which was included in the culture medium, was found to contaminate the lysate fraction prepared from dying cells, giving rise to difficulty with accurate protein quantitation. We thus ran the following experiments not on the 'protein' basis but on the 'cell number' basis.

Sphingosine treatment evoked a dramatic loss of the classical enzymatic cross-linking activity of TGase in the cell lysate. As shown in Fig. 2A, we observed ≈60% loss of the activity as early as

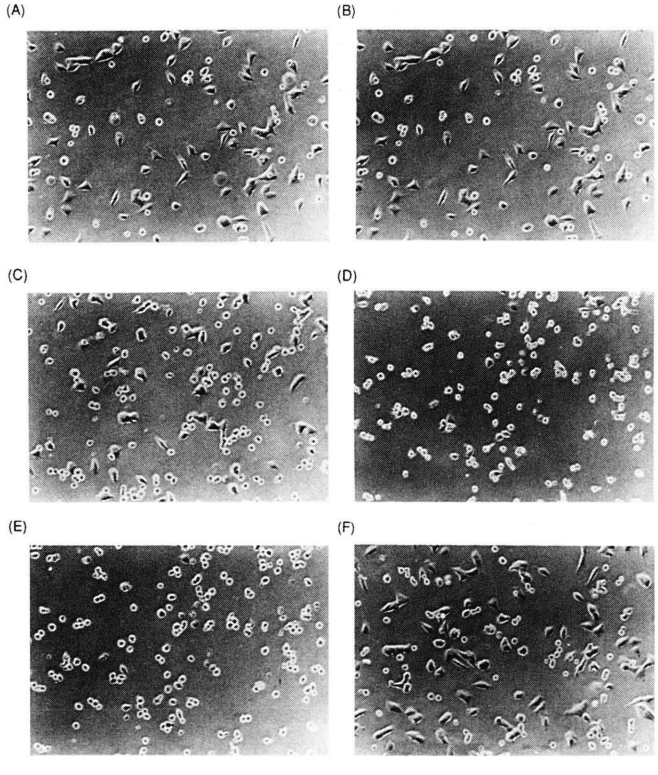

Fig. 1. Cytotoxic effect of sphingosine on A375–S2 cells. Cells ($5x10^5$ per flask) were seeded in 25 cm^2 flasks. After overnight culture, cells were treated with 40 μM sphingosine. The morphology of cells was photographed at 2 min (C), 7 min (D) and 15 min (E), demonstrating rapid and severe damage to the cells caused by the lipid. Twenty minutes after the treatment, most cells became detached from the flask and floating in the medium, while cells with no treatment (A) and vehicle (bovine albumin / methanol) treatment (B) retained their normal, healthy morphology at 21 min (F).

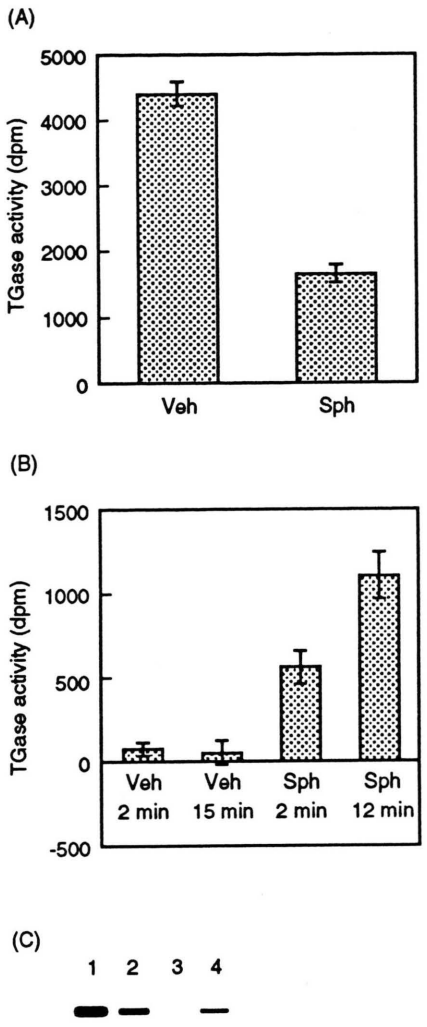

Fig. 2. Linkage of sphingosine-induced death of A375–S2 cells with redistribution of tissue type TGase to the cell nuclei. Cells ($4.6x10^6$ per flask) were seeded in 225 cm^2 flasks and cultured overnight. After sphingosine treatment, cells were collected and washed, while the culture media were saved. Cell nuclei were then separated from lysate fractions as described in Materials and Methods. (A) TGase activity of the cell lysate was assayed after 2 min sphingosine treatment (Sph), at which time point most cells remained attached to the flask, though morphological abnormality was apparent in some cells (Fig. 1C). Lysate from cells with vehicle treatment (Veh) was similarly assayed for TGase activity. (B) Culture media were recovered from cells that received sphingosine treatment for 2 min and 12 min, and then the post-GTP column preparations were assayed for TGase activity. Media recovered 2 min and 15 min after vehicle treatment were similarly assayed for TGase activity. Values shown in (A) and (B) were expressed as mean ± SEM of duplicate determinations from each of two equivalent experiments. In (B), the basal TGase activity of fresh medium origin was subtracted. (C) Nuclear fractions (lanes 3 and 4) and lysate fractions (lanes 1 and 2) were prepared from cells with 2 min sphingosine treatment (lanes 2 and 4) and from cells with vehicle treatment (lanes 1 and 3). Electrophoretic separation of these fractions was followed by immunoblotting with anti-tissue TGase antibody CUB74, which detected a protein band at its migration rate of 77 kDa. See Materials and Methods for experimental detail, and note that all of the data shown here were expressed on the 'cell number' basis rather than on the 'protein' basis, as described in Results and Discussion.

2 min after the addition of sphingosine. This activity loss was partly explained by the concomitant emergence of the activity in the culture medium (Fig. 2B), suggesting a leak of the TGase activity from the damaged cells. Such a loss of TGase from the cells was further confirmed by immunoblotting analysis using CUB 74, a monoclonal antibody widely used to detect tissue type TGase (Takaku *et al.*, 1995; Birckbichler *et al.*, 1985; Ohashi *et al.*, 1995) (compare the 77 kDa band between lanes 1 and 2, Fig. 2C).

To our surprise, however, we found that a small, but significant portion of tissue TGase was redistributed to the nucleus by the sphingosine treatment (note the 77 kDa band appeared in lane 4, Fig. 2C, but not in lane 3 for the mock sample). In some experiments, a second 60 kDa band also emerged in the nuclear fraction as well, presumably suggesting a nuclear distribution of a proteolytic degradate of the enzyme during sphingosine-induced cell death (data not shown).

DNA hydrolytic activity of tissue TGase

Knowing i) the sphingosine-induced nuclear redistribution of tissue TGase (Fig. 2C), ii) the implication of the enzyme in cell death (Piacentini *et al.*, 1991; Piacentini and Autuori, 1994; Amendola *et al.*, 1996; Zhang *et al.*, 1995; Szondy *et al.*, 1997), iii) that the enzyme is also associated with a hy-

Table I. Lactate dehydrogenase assay of sphingolipid-treated A375–S2 cells.

Treatment	OD_{570}	Net absorbance (% Cell survival)
Control (no cells)	0.177 ± 0.012	–
No treatment	0.577 ± 0.091	0.400 ± 0.079 (100.0 ± 19.8)
Mock (vehicle)	0.553 ± 0.047	0.376 ± 0.035 (93.9 ± 8.8)
D–Sphingosine	0.354 ± 0.028	0.177 ± 0.016 (44.3 ± 2.5)
DL–Sphinganine	0.433 ± 0.024	0.256 ± 0.012 (64.0 ± 3.0)

Note. A375–S2 cells were seeded on a 96–well plate ($3x10^3$ per well). After overnight culture in RPMI 1640 medium containing 10% fetal calf serum, cells received no treatment, vehicle for sphingolipid, 40 μM sphingosine and 40 μM sphinganine. Seven minutes later, medium was aspirated, and cells were washed with phosphate-buffered saline (100 μl x 2) and were then lyzed with 0.1% Tween 20. Lactate dehydrogenase activity retained in the cells was assayed according to the manufacturer's protocol. Values are expressed as mean ± SEM of triplicate determinations.

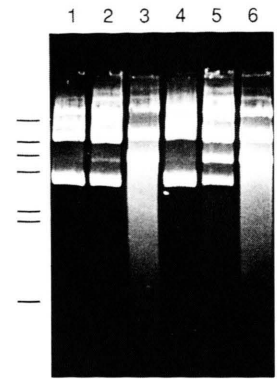

Fig. 3. Association of tissue TGase with DNA hydrolytic activity. Purified guinea pig liver TGase was examined for DNA hydrolytic activity. In the presence of Mg^{2+}, TGase caused random cleavage of a plasmid DNA (lane 6). Zn^{2+} (lane 5), but not Ca^{2+} (lane 3), abrogated this activity of TGase. No DNA degradation was observed in the absence of the enzyme (lane 1) or in the presence of boiled enzyme (data not shown). Neither Zn^{2+} (lane 4) nor Ca^{2+} (lane 2) caused DNA hydrolysis, if merely mixed with the plasmid in the absence of the enzyme. Bars on the left indicate size markers, Hind III digest of λ phage DNA (Gibco-BRL, MD); from the top, 23.1, 9.4, 6.6, 4.4, 2.3, 2.0 and 0.56 kb.

drolytic activity (Folk and Chung, 1985; Parameswaran *et al.*, 1997), and iv) that DNA damage is a hallmark of cell death, we tested a purified preparation of guinea pig liver TGase, a canonical tissue TGase, for DNA hydrolytic activity. A pGEM-3Z plasmid gave its smeared pattern on agarose electrophoresis after incubation with TGase (lane 6, Fig. 3), indicating random cleavage of DNA that is reminiscent of necrotic, but not apoptotic, cell death (Gerschenson and Rotello, 1992). This DNA hydrolytic activity was dependent on tissue TGase, since no DNA degradation was observed in the absence of the enzyme (lane 1) or in the presence of heat-inactivated enzyme (data not shown). Furthermore, no DNA hydrolysis was observed, if Mg^{2+} was omitted (data not shown). The Mg^{2+} requirement is reminscent of the GTP/ATP hydrolytic activity of tissue TGase (Lee *et al.*, 1989; Takeuchi *et al.*, 1992; Lee *et al.*, 1993; Takeuchi *et al.*, 1994). Interestingly, the DNA degradation was essentially inhibited by Zn^{2+} (lane 5), but not by Ca^{2+} (lane 3), an indispensable co-factor for the classical cross-linking activity of TGase (Folk and Finlayson, 1977; Lorand and Conrad, 1984; Chung, 1975; Greenberg *et al.*, 1991; Folk, 1980; Aeschlimann *et al.*, 1994; Margosiak *et al.*, 1990; Ando *et al.*, 1989). Ca^{2+} or Zn^{2+}, when tested alone in the absence of the enzyme, did not exhibit any observable effect (lanes 2 and 4).

Although many reports have implicated that tissue type TGase plays a role in cell death, particularly in apoptotic cell death, the implication is mostly considered in terms of the classical cross-linking activity of the enzyme located in the cytoplasm (Greenberg *et al.*, 1991). However, the nuclear localization of tissue TGase observed in A375-S2 cells that underwent sphingolipid-induced cell death (Fig. 2C) strongly suggests a novel aspect of the enzyme, when combined with its DNA hydrolytic activity (Fig. 3). It may be mentioned that the preexisting TGase, but not newly synthesized enzyme, was presumably relocated into the cell nuclei, since this relocation occurred in cells at an early phase (in 2 min after sphingosine treatment, Fig. 2C) of death process.

Moreover, the DNA hydrolytic activity shown in Fig. 3 expands the functional repertoire of tissue TGase, which has been hitherto reported to act as a classical cross-linking enzyme (Folk and Finlayson, 1977; Lorand and Conrad, 1984; Chung, 1975; Greenberg *et al.*, 1991; Folk, 1980; Aeschlimann *et al.*, 1994; Margosiak *et al.*, 1990; Ando *et al.*, 1989), as a nucleotide hydrolase (Achyuthan and Greenberg, 1987; Lee *et al.*, 1989; Takeuchi *et al.*, 1992; Lee *et al.*, 1993; Monsonego *et al.*, 1997; Lai

et al., 1996; Singh and Cerione, 1996; Takeuchi *et al.*, 1994) and as a G protein in certain cell signaling systems (Nakaoka *et al.*, 1994; Chen *et al.*, 1996; Feng *et al.*, 1996). The DNA hydrolytic activity of tissue TGase is also reminiscent of p53, a pivotal component in apoptotic cell death, which has recently been reported to exert an exonuclease activity (Mummenbrauer *et al.*, 1996).

Achyuthan K. E. and Greenberg C. S. (1987), Identification of a guanosine triphosphate-binding site on guinea pig liver transglutaminase. Role of GTP and calcium ions in modulating activity. J. Biol. Chem. **262,** 1901–1906.

Aeschlimann D. and Paulsson M. (1994), Transglutaminases: protein cross-linking enzymes in tissues and body fluids. Thromb. Haemost. **71,** 402–415.

Amendola A., Gougeon M.-L., Poccia F., Bondurand A., Fesus L. and Piacentini M. (1996), Induction of "tissue" transglutaminase in HIV pathogenesis: evidence for high rate of apoptosis of CD4+T lymphocytes and accessory cells in lymphoid tissues. Proc. Natl. Acad. Sci. USA **93,** 11057–11062.

Ando H., Adachi M., Umeda K., Matsuura A., Nonaka M., Uchio, R., Tanaka H. and Motoki M. (1989), Purification of Characteristics of a Novel Transglutaminase Derived from Microorganisms. Agric. Biol. Chem. (Tokyo) **53,** 2613–2617.

Bates R. C., Buret A., van Helden D. F., Horton M. A. and Burns G. F. (1994), Apoptosis induced by inhibition of intercellular contact. J. Cell Biol. **125,** 403–415.

Birckbichler P. J., Orr G. R., Patterson Jr. M. K., Conway E. and Carter H. A (1981), Increase in proliferative markers after inhibition of transglutaminase. Proc. Natl. Acad. Sci. USA. **78,** 5005–5008.

Birckbichler P. J., Upchurch H. F., Patterson M. K. Jr. and Conway E. (1985), A monoclonal antibody to cellular transglutaminase. Hybridoma **4**, 179–186.

Bradford M. (1976), A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72,** 248–254.

Chen S., Lin F., Iismaa S., Lee K. N., Birckbichler P. J. and Graham R. M. (1996), Alpha 1-adrenergic receptor signaling via Gh is subtype specific and independent of its transglutaminase activity. J. Biol. Chem. **271,** 32385–32391.

Chung S. I. (1975), Multiple molecular forms of transglutaminases in human and guinea-pig. Isozymes **1** (Markert C. L. ed.), Academic Press, New York, pp. 259–274.

Decker T. and Lohmann-Matthes M.-L. (1988), A quick and simple method for the quantitation of lactate dehydrogenase release in measurements of cellular cytotoxicity and tumor necrosis factor (TNF) activity. J. Immunol. Methods **115,** 61–69.

Eitan S. and Schwartz M. (1993), A transglutaminase that converts interleukin-2 into a factor cytotoxic to oligodendrocytes. Science **261,** 106–108.

Eitan S., Solomon A., Lavie V., Yoles E., Hirschberg D. L., Belkin M. and Schwartz M. (1994), Recovery of visual response of injured adult rat optic nerves treated with transglutaminase. Science **264,** 1764–1768.

Feng J.-F., Rhee S. G. and Im M.-J. (1996), Evidence that phospholipase delta1 is the effector in the Gh (transglutaminase II)-mediated signaling. J. Biol. Chem. **271,** 16451–16454.

Folk J. E. and Finlayson J. S. (1977), The epsilon-(gamma-glutamyl) lysine crosslink and the catalytic role of transglutaminases. Adv. Protein Chem. **31,** 1–133.

Folk J. E. (1980), Transglutaminases. Annu. Rev. Biochem. **49,** 517–531.

Folk J. E. and Chung S. I. (1985), Transglutaminases. Methods in Enzymol. **113** (Meister A. ed.) Academic Press, New York, pp. 358–375.

Gerschenson L. E. and Rotello R. J. (1992), Apoptosis: a different type of cell death. FASEB J. **6,** 2450–2455.

Greenberg C. S., Birckbichler P. J. and Rice R. H. (1991), Transglutaminases: multifunctional cross-linking enzymes that stabilize tissues. FASEB J. **5,** 3071–3077.

Hannun Y. A. (1996), Functions of ceramide in coordinating cellular responses to stress. Science **274,** 1855–1859.

Hauser J. M. L., Buehrer B. M. and Bell R. M. (1994), Role of ceramide in mitogenesis induced by exogenous sphingoid bases. J. Biol. Chem. **269,** 6803–6809.

Knight C. R. L., Rees R. C. and Griffin M. (1991), Apoptosis: a potential role for cytosolic transglutaminase and its importance in tumour progression. Biochim. Biophys. Acta **1096,** 312–318.

Kolesnick R. and Golde D. W. (1994), The sphingomyelin pathway in tumor necrosis factor and interleukin-1 signaling. Cell **77,** 325–328.

Korzeniewski C. and Callewaert D. M. (1983), An enzyme-release assay for natural cytotoxicity. J. Immunol. Methods **64,** 313–320.

Lai T.-S., Slaughter T. F., Koropchak C. M., Haroon Z. A. and Greenberg C. S. (1996), C-terminal deletion of human tissue transglutaminase enhances magnesium-dependent GTP/ATPase activity. J. Biol. Chem. **271,** 31191–31195.

Lee K. N., Birckbichler P. J. and Patterson Jr. M. K. (1989), GTP hydrolysis by guinea pig liver transglutaminase. Biochem. Biophys. Res. Commun. **162,** 1370–1375.

Lee K. N., Arnold S. A., Birckbichler P. J., Patterson Jr. M. K., Fraij B. M., Takeuchi Y. and Carter H. A. (1993), Site-directed mutagenesis of human tissue transglutaminase: Cys-277 is essential for transglutaminase activity but not for GTPase activity. Biochim. Biophys. Acta **1201**, 1–6.

Lorand L., Campbell-Wilkes L. K. and Cooperstein L. (1972), A filter paper assay for transamidating enzymes using radioactive amine substrates. Anal. Biochem. **50,** 623–631.

Lorand L. and Conrad S. M. (1984), Transglutaminases. Mol. Cell. Biochem. **58,** 9–35.

Margosiak S. A., Dharma A., Bruce-Carver M. R., Gonzales A. P., Louie D. and Kuehn D. (1990), Identification of the large subunit of ribulose-1, 5-bisphosphate carboxylase-oxygenase as a substrate for transglutaminase in *Medicago-sativa* L. Alfalfa. Plant Physiol. **92,** 88–96.

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.

Moore G. E., Gerner R. E. and Franklin H. A. (1967), Culture of normal human leukocytes. J. Am. Med. Assoc. **199,** 519–524.

Mummenbrauer T., Janus F., Müller B., Wiesmüller L., Deppert W. and Grosse F. (1996), p53 protein exhibits 3'-to-5' exonuclease activity. Cell **85,** 1089–1099.

Murtaugh M. P., Mehta K., Johnson J., Myer M., Juliano R. L. and Davies P. J. A. (1983), Induction of tissue transglutaminase in mouse peritoneal macrophages. J. Biol. Chem. **258,** 11074–11081.

Nakai S., Mizuno K., Kaneta M. and Hirai Y. (1988), A simple, sensitive bioassay for the detection of interleukin-1 using human melanoma A375 cell line. Biochem. Biophys. Res. Commun. **154,** 1189–1196.

Nakaoka H., Perez D. M., Baek K. J., Das T., Husain A., Misono K., Im M.-J. and Graham R. M. (1994), Gh: a GTP-binding protein with transglutaminase activity and receptor signaling function. Science **264,** 1593–1596.

Ohashi H., Itoh Y., Birckbichler P. J. and Takeuchi Y. (1995), Purification and characterization of rat brain transglutaminase. J. Biochem. (Tokyo) **118,** 1271–1278.

Parameswaran K. N., Cheng X.-F., Chen E. C., Velasco P. T., Wilson J. H. and Lorand L. (1997), Hydrolysis of gamma:epsilon isopeptides by cytosolic transglutaminases and by coagulation factor XIIIa. J. Biol. Chem. **272,** 10311–10317.

Piacentini M., Fesus L., Farrace M. G., Ghibelli L., Piredda L. and Melino G. (1991), The expression of "tissue" transglutaminase in two human cancer cell lines is related with the programmed cell death (apoptosis). Eur. J. Cell Biol. **54,** 246–254.

Piacentini M. and Autuori F. (1994), Immunohistochemical localization of tissue transglutaminase and Bcl-2 in rat uterine tissues during embryo implantation and post-partum involution. Differentiation **57,** 51–61.

Singh U. S. and Cerione R. A. (1996), Biochemical effects of retinoic acid on GTP-binding protein/transglutaminases in HeLa cells. Stimulation of GTP-binding and transglutaminase activity, membrane association, and phosphatidylinositol lipid turnover. J. Biol. Chem. **271,** 27292–27298.

Spiegel S. and Merrill Jr. A. H. (1996), Sphingolipid metabolism and cell growth regulation. FASEB J. **10,** 1388–1397.

Szondy Z., Molnar P., Nemes Z., Boyiadzis M., Kedei N., Tóth R. and Fesus L. (1997), Differential expression of tissue transglutaminase during *in vivo* apoptosis of thymocytes induced via distinct signalling pathways. FEBS Lett. **404,** 307–313.

Takaku K., Futamura M., Saitoh S. and Takeuchi Y. (1995), Tissue-type transglutaminase is not a tumor-related marker. J. Biochem. (Tokyo) **118,** 1268–1270.

Takeuchi Y., Birckbichler P. J., Patterson Jr. M. K. and Lee K. N. (1992), Putative nucleotide binding sites of guinea pig liver transglutaminase. FEBS Lett. **307,** 177–180.

Takeuchi Y., Birckbichler P. J., Patterson Jr. M. K., Lee K. N. and Carter H. A. (1994), Calmodulin regulates nucleotide hydrolysis activity of tissue transglutaminase. Z. Naturforsch. **49c,** 453–457.

Zhang L.-X., Mills K. J., Dawson M. I., Collins S. J. and Jetten A. M. (1995), Evidence for the involvement of retinoic acid receptor RAR alpha-dependent signaling pathway in the induction of tissue transglutaminase and apoptosis by retinoids. J. Biol. Chem. **270,** 6022–6029.