Effects of Tissue Transglutaminase on Retinoic Acid-induced Cellular Differentiation and Protection against Apoptosis*

Marc A. Antonyak‡, Ugra S. Singh§, David A. Lee‡, Jason E. Boehm‡, Carolyn Combs‡, Marsha M. Zgola¶, Rodney L. Page¶, and Richard A. Cerione‡¶

From the ‡Departments of Molecular Medicine and §Clinical Sciences, Cornell University, Ithaca, New York 14853 and the ¶Department of Molecular Cardiology, Cardiovascular Research Institute, Texas A&M University, Veterans Affairs Hospital, Temple, Texas 76504

Retinoic acid (RA) and its various synthetic analogs affect mammalian cell growth, differentiation, and apoptosis. Whereas treatment of the human leukemia cell line HL60 with RA results in cellular differentiation, addition of the synthetic retinoid, N-(4-hydroxyphenyl) retinamide (HPR), induces HL60 cells to undergo apoptosis. Moreover, pretreatment of HL60 cells as well as other cell lines (i.e. NIH3T3 cells) with RA blocks HPR-induced cell death. In attempting to discover the underlying biochemical activities that might account for these cellular effects, we found that monodansylcadaverine (MDC), which binds to the enzyme (transamidase) active site of tissue transglutaminase (TGase), eliminated RA protection against cell death and in fact caused RA to become an apoptotic factor, suggesting that the ability of RA to protect against apoptosis is linked to the expression of active TGase. Furthermore, it was determined that expression of exogenous TGase in cells exhibited enhanced GTP binding and transamidation activities and mimicked the survival advantage imparted by RA. We tested whether the ability of this dual function enzyme to limit HPR-mediated apoptosis was a result of the ability of TGase to bind GTP and/or catalyze transamidation and found that GTP binding was sufficient for the protective effect. Moreover, excessive transamidation activity did not appear to be detrimental to cell viability. These findings, taken together with observations that the TGase is frequently up-regulated by environmental stresses, suggest that TGase may function to ensure cell survival under conditions of differentiation and cell stress.

Tissue transglutaminase II (TGase) is a member of a family of closely related thiol enzymes that are derived from a common ancestor (1, 2). The TGases function as calcium-dependent acyl transferases that catalyze the formation of an amide bond between γ-carboxamide groups of peptide-bound glutamine residues and either the ε-amino groups of lysine residues in proteins or the amino groups from polyamines. This enzymatic activity has been implicated in a number of cellular processes including cell attachment, bone development, axonal growth and regeneration, as well as cell growth regulation and apoptosis (3–10). The possible role of polyamines as amino group donors for the TGase enzymatic (transamidation) activity may hold important significance for cell growth regulation (11, 12), and the covalent linkage to proteins may represent a key regulatory step (2).

The TGase is especially interesting because of its ability to bind and hydrolyze GTP-like traditional signaling GTP-binding proteins (G proteins) (13–15). A particularly well studied example involves the α1-adrenergic receptor-mediated stimulation of phospholipase C (PLC) activity (most likely PLC-δ, Ref. 16), where it was originally shown that a 70–80-kDa GTP-binding protein, Gh, was responsible for coupling α1-adrenergic agonists to the stimulation of phosphoinositide lipid metabolism (17). Various studies have also suggested that the GTP binding/GTP hydrolytic activity of the TGase is closely linked to the enzymatic transamidation activity, such that the enzyme activity is off when the TGase is in the GTP-bound but not the GDP-bound state (14, 18). However, the nature of the interplay between guanine nucleotide binding and transamidation activity remains controversial, as other studies have suggested that both GTP and GDP inhibit the enzyme (19).

It has been well documented that TGase activity is tightly coupled to the effects of retinoic acid (RA) in various cell types (6–9). In some cases, both expression and activation of the GTP binding and transamidation activities of the TGase are stimulated by RA treatment; however, in HeLa cells we found that RA promotes the activation of the TGase without having any effect on TGase expression (20). The cellular effects of RA are mediated via nuclear receptor interactions (21, 22). Six retinoid receptors have been identified and can be divided into two classes, the RARs and the RXRs (each containing α, β, and γ subtypes). All trans-retinoic acid, designated simply as RA, activates RAR-RXR heterodimers, whereas 9-cis-retinoic acid activates both RAR-RXR heterodimers and RXR homodimers. These nuclear receptors function as transcriptional activators that bind to specific response elements located in the promoters of several genes.

Because of its growth inhibitory activity, RA has been examined for potential use in the treatment of human cancers. However, the natural retinoids have shown only limited activity against most cancers, which has prompted efforts to examine the effects of synthetic analogs of the retinoids. One such analog, all-trans-N-(4-hydroxyphenyl)retinamide (fenretinamide

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† To whom correspondence should be addressed: Dept. of Molecular Medicine, VMC, Cornell University, Ithaca, NY 14853-6401. Tel.: 607-253-3658; Fax: 607-253-3665; E-mail: rac1@cornell.edu.

The abbreviations used are: TGase, tissue transglutaminase; RA, retinoic acid; HPR, N-(4-hydroxyphenyl) retinamide; MDC, monodansylcadaverine; GTP, guanosine triphosphate; PLC, phospholipase C; GDP, guanosine diphosphate; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; AMP-PNP, adenosine 5′-[(β,y-mimino)triphosphate]; Z-DEVD-FMK, benzoyloxycarbonyl-DEVD-fluoromethylketone; WT, wild type; RAR, retinoic acid receptor; RXR, retinoid X receptor; HA, hemagglutinin; TBST, Tris-buffered saline Tween.
or HPR), has received a significant amount of attention because of its promise in the treatment of breast and prostate cancers as well as other human malignancies (23–26). Although it has been suggested that HPR action is mediated via retinooid receptors (27), it is interesting that HPR appears to be consistently associated with apoptosis, whereas RA has been reported to give rise to both cellular differentiation and apoptosis (28, 29).

In this study, we set out to examine the actions of RA and HPR on the leukemia cell line HL60 and the fibroblast cell line NIH3T3. We show that HPR but not RA induces an apoptotic response in both cell types. Pretreatment of HL60 or NIH3T3 cells with RA affords a strong protection against HPR-induced apoptosis, and this protective effect of RA can be correlated with the expression of active TGase. The binding of an active site-directed inhibitor, monodansylcadaverine (MDC), not only eliminated the ability of RA to inhibit HPR-mediated apoptosis, but also turned RA into a potent death factor. To elucidate which aspect of TGase function is important for the survival advantage, we generated mutant forms of TGase that were defective in GTP binding, transamidation, or both activities and tested the ability to promote cell viability. Our data indicate that the GTP binding activity, rather than transamidation activity, may be crucial for the survival response of the TGase.

**EXPERIMENTAL PROCEDURES**

**Materials**—Caspase inhibitor II (Z-DEVD-FMK) and ionomycin were purchased from CalBiochem, and caspase 7 was obtained from BD PharMingen. RA, HPR, and MDC were obtained from Sigma. The TGase monoclonal antibody was from Neomarkers, and the anti-HA site-directed mutagenesis kit (Stratagene) was used to introduce single site-directed mutagenesis into the expression plasmid. As a control for transamidation activity, a monoclonal antibody was from Covance. The TGase monoclonal antibody was from Neomarkers, and the anti-HA monoclonal antibody was from Covance. \(\text{[2-3P]GTP\)} was purchased from PerkinElmer Life Sciences, and all other materials were from Fisher unless otherwise stated.

**Cell Culture**—HL60 cells were maintained in RPMI 1640 medium containing 10% fetal bovine serum and 100 units/ml penicillin, and NIH3T3 cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% calf serum with 100 units/ml penicillin. Both cell lines were maintained in a humidified atmosphere with 5% CO\(_2\) at 37 °C. For the various treatments involving retinooids, the cells were grown to subconfluence in medium containing 10% serum, and then medium containing 1.5% serum with 5 \(\mu\)M RA or HPR or 50 \(\mu\)M MDC was added for the times indicated under “Results and Discussion.” Cells were rinsed with phosphate-buffered saline and then lysed with cell lysis buffer (10 mM Na\(_2\)HPO\(_4\), 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% SDS, 0.2% Na\(_3\)citrate, 0.004% NaF, 1 mM NaVo\(_4\), 25 mM \(\beta\)-glycerophosphoric acid, 100 \(\mu\)g/ml phenylmethylsulfonyl fluoride, and 1 \(\mu\)g/ml each aprotinin and leupeptin, pH 7.35). The lysates were clarified by centrifugation at 12,000 \(\times\) g for 10 min at 4 °C. Protein concentrations were determined using the Bio-Rad DC protein assay.

**Western Blot Analysis**—The total cell lysate (60 \(\mu\)g) of each sample was combined with Laemmli sample buffer, boiled for 5 min, and subjected to SDS-polyacrylamide gel electrophoresis (PAGE) on 7.5% acrylamide gels for resolution of each protein analyzed. The proteins were transferred to nitrocellulose filters and blocked for 1 h with TBST (20 mM Tris, 137 mM NaCl, pH 7.4, and 0.02% Tween 20) containing 5% nonfat dry milk. The filters were incubated with either anti-TGase, anti-actin, or anti-HA primary antibody diluted 1:1000 in TBST for 2 h at room temperature, then washed three times with TBST at 5-min intervals. To detect the primary antibodies, anti-mouse or anti-rabbit conjugated to horseradish peroxidase (Amersham Pharmacia Biotech) diluted 1:5000 in TBST was then incubated with the filters for 1 h, followed by three TBST washes. The protein bands were visualized on x-ray film after exposing the filters to chemiluminescence reagent (ECL, Amersham Pharmacia Biotech).

**Photoaffinity Labeling of the TGase**—Photoaffinity labeling of the TGase was performed by incubating 60 \(\mu\)g of whole cell lysates with 3 \(\mu\)Ci of \(\text{[125I]GTP\)} in 50 mM Tris-HCl, pH 7.4, 2 mM EGTA, 1 mM dithiothreitol, 20% (v/v) glycerol, 100 mM NaCl, and 500 \(\mu\)M AMP-PNP for 10 min at room temperature. The samples were placed in an ice bath and irradiated with UV light (254 nm) for 15 min, mixed with 5 \(\times\) Laemmli sample buffer, and boiled for 5 min. SDS-PAGE was performed, followed by transfer to nitrocellulose filters and exposure on x-ray film.

**Transamidation Reactions**—Transglutaminase activity was measured as the Ca\(^{2+}\)-dependent incorporation of \(\text{[\text{H}]putrescine (Amer-}

Pharmacia Biotech\)} into N,N-dimethylethylene. Whole cell lysates (175 \(\mu\)g) were added to a reaction mix consisting of 25 mM HEPES, pH 7.4, 100 mM CaCl\(_2\), 5 mM KCl, 0.3 mM NaHPO\(_4\), 1 mM NaHCO\(_3\), 5 mM glucose, 1 mM CaCl\(_2\), 1 mg/ml dimethylethylene, 20 mM dithiothreitol, and 250 \(\mu\)M \(\text{[\text{H}]putrescine (0.5 \mu\text{Ci\)} in a final volume of 500 \(\mu\)l. The reaction mixtures were incubated at room temperature for 30 min, and then stopped by the addition of 100 \(\mu\)l of 50% trichloroacetic acid. The suspension was centrifuged at 12,000 \(\times\) g for 30 min and the precipitates were washed twice with 10% trichloroacetic acid and then dissolved in 100 \(\mu\)l of 1 N NaOH. The rate of incorporation was measured by liquid scintillation counting, and the transamidation activity was expressed as fold activation over control samples.

**DNA Fragmentation Assay**—For assessment of DNA fragmentation, cell pellets of samples treated with 5 \(\mu\)M RA or HPR for various lengths of time were solubilized in 100 \(\mu\)l of 10 mM Tris-Cl, pH 7.4, containing 10 mM NaCl, 20 mM EDTA, and 1% Triton X-100 for 30 min at 4 °C. The suspension was then centrifuged at 12,000 \(\times\) g for 15 min. To the supernatant (90 \(\mu\)l), 10 \(\mu\)l of 10% SDS and 5 \(\mu\)l of a 10 mg/ml stock of proteinase K were added and incubated for 3 h at 50 °C. DNA was purified from solution with phenol/chloroform and then was dissolved in TE (40 mM Tris acetate and 1 mM EDTA). Equal amounts of sample were resolved on a 1.2% agarose gel and visualized by ethidium bromide staining.

**Nuclear Condensation or Blebbing Assay**—Cells were seeded on coverslips in 6-well dishes and grown in complete medium for 2–3 days. The cells were then incubated in medium containing 5 \(\mu\)M RA or HPR with or without 50 \(\mu\)M MDC for different times. The cultures were then fixed and stained with 4,6-diamidino-2-phenylindole (2 \(\mu\)g/ml) for viewing by fluorescence microscopy. Apoptotic cells were identified by condensed nuclei and/or blebbing.

**Construction of Mutant TGase Expression Vectors**—The QuikChange site-directed mutagenesis kit (Stratagene) was used to introduce single amino acid substitutions within the GTP binding and active site domains of the TGase. A tetracycline inducible vector containing HA-tagged TGase (pTRE-WT-TGase) was used as the template for each mutagenesis reaction, and the following sets of primers were used to generate mutant serine 171 \(\rightarrow\) cysteine (S171C), 5'-GGCTTATTACCAAGGTCGTGCCAAAGTGCTGAACAGAC and 3'-GGTTCTTGATGAACTTGGCCTCGCAGCCCTGGTAGCTAGAAGCC; serine 171 glutamic acid (S171E), 5'-GGCTTATTACCAAGGTCGTGCCAAAGTGCTGAACAGAC and 3'-GTGGTTCTGTAGAATCTTGCCCGCTCGCAGCCCTGGTAGCTAGAAGCC; glycine 165 \(\rightarrow\) cysteine (G165C), 5'-GTCTTACAGCTGAGCCACTGCTGTCGCTGGAAGCC and cysteine 277 \(\rightarrow\) valine (C277V), 5'-GTCAGATGCGCATGCAGAGCTGCTGTCGCTGGAAGCC and cysteine 277 \(\rightarrow\) glycine (C277G). Each construct was sequenced to confirm the presence of the mutation.

**RESULTS AND DISCUSSION**

The overall goal of these studies was to better understand the nature of the interplay between RA and its synthetic analog, HPR, in normal and cancer cell lines. Although it was originally suspected that these retinooids would bind common receptors and potentially influence the same signaling pathways, various studies have suggested that they in fact mediate distinct biological outcomes (28, 29). This is perhaps best exemplified in the human leukemia cell line HL60, where RA and HPR elicit markedly different effects; however, various other cell lines including the mouse fibroblast cell line NIH3T3 also show striking differences between RA and HPR treatments.

Fig. 1A shows that when HL60 cells were exposed to HPR for various periods of time a strong apoptotic response was observed as gauged by nuclear condensation or blebbing. HPR-induced apoptosis in HL60 cells started within 6–8 h of treatment, and nearly 95% of the cells were apoptotic by 48 h. To confirm that these HPR-treated cells were undergoing programmed cell death, an additional apoptotic assay that tests the integrity of the genomic DNA isolated from the cells was also performed. Consistent with cells undergoing programmed cell death, cytoplasmic DNA isolated from HL60 cells treated with HPR was evident and fragmented, as indicated by a resultant smear of the ethidium bromide staining.
treated with HPR. The cells were processed as explained in
Experimental Procedures, calculating the ratio of apoptotic to nonapoptotic cells. The
apoptotic response was specific for the synthetic retinoid RA and NIH3T3 cells incubated with HPR also induced a
apoptotic response as visualized by DNA fragmentation was
observed for HL60 cells treated with the two retinoids. As seen in
Fig. 1, HL60 and NIH3T3 cells were treated with 5 μM RA or
HPR for up to 72 h and then scored for programmed cell death as identified by nuclear condensation and/or blebbing as described under “Experimental Procedures.” Cells with condensed nuclei were classified as apoptotic, and the percentage of cell death was determined by calculating the ratio of apoptotic to nonapoptotic cells. The inset is of a
DNA fragmentation assay that was also performed on HL60 cells treated with HPR. The cells were processed as explained in “Experimental Procedures,” and the extracted DNA was resolved on an agarose gel and visualized with ethidium bromide.

(Fig. 1A, inset). In contrast, treatment of HL60 cells with RA for up to 72 h resulted in no detectable cell death, verifying that the apoptotic response was specific for the synthetic retinoid and consistent with previous findings that RA causes HL60 cells to undergo differentiation rather than programmed cell death (6–9). We investigated whether an additional cell type might also produce physiological outcomes similar to those observed for HL60 cells treated with the two retinoids. As seen in
Fig. 1B, NIH3T3 cells incubated with HPR also induced a
cell death response, but with slower kinetics than observed in
HL60 cells (compare Fig. 1, A and B). Only about 20% of the
cells were apoptotic after 18 h of HPR treatment and it took 72 h of exposure to HPR to produce an apoptotic rate of ~90%. Consistent with RA stimulation of HL60 cells, incubation of NIH3T3 cells with RA for up to 72 h did not enhance cell death.

We further examined the basis of HPR-induced apoptosis in HL60 cells using inhibitors against caspases and histone deacetylase. The rationale was to determine whether this apoptotic response utilized the caspase-mediated proteolysis typically involved in apoptotic responses (30, 31), or whether HPR-induced apoptosis might also require the inhibition of gene expression mediated through the deacetylation of histones. We found that treatment of HL60 cells with the histone deacetylase inhibitor, trichostatin A, had no effect on HPR-stimulated DNA fragmentation, whereas addition of the caspase inhibitor, Z-DEVD-FMK, caused a marked reduction in DNA fragmenta-

Fig. 2. Inhibition of caspase activity suppresses HPR-induced apoptosis. HL60 cells were grown to subconfluence in complete growth medium, and then the cells were treated with the histone deacetylase inhibitor, trichostatin A at 100 ng/ml or the caspase inhibitor, Z-DEVD-FMK, at 100 nM ± 18 h of HPR treatment. The cells from each sample were pelleted, DNA-extracted, and analyzed for DNA fragmentation. Lanes 4 and 5 and lanes 7 and 8 represent duplicate conditions.

Fig. 3. Pretreatment of HL60 cells with RA blocks the apoptotic effects of HPR. Cells were treated with 5 μM RA for 48 h, rinsed, and then resuspended in fresh medium containing 5 μM HPR. At the times indicated, the DNA was extracted from the treated cells and analyzed for fragmentation following gel electrophoresis and ethidium bromide staining.

During cell differentiation, an important question concerns how cells are able to maintain cell cycle arrest without undergoing cell death. This raises the possibility that during differentiation it is necessary to activate mechanisms that provide protection against apoptosis. Given that the two retinoids, RA and HPR, appear to input into distinct cellular programs such that RA induced differentiation while HPR elicited an apoptotic response, it was interesting to examine whether RA might be able to stimulate a cellular activity that protects against HPR-mediated cell death. Fig. 3 shows that when HL60 cells were pretreated with RA for 2 days and then exposed to HPR for various times up to 24 h, the ability of HPR to stimulate an apoptotic response as visualized by DNA fragmentation was nearly eliminated. RA exhibited a very similar protective effect against HPR-promoted apoptosis of the fibroblast cell line NIH3T3 and the breast cancer cell line SKBR3 (data not shown). In all cases maximal protection from HPR-induced cell death required pretreating the cells with RA for 2 days. This effect elicited by RA can be distinguished from the apoptotic effects of HPR, which occur within 6–12 h of exposure of HL60 cells to the synthetic retinoid, thereby suggesting that the protective effects by RA are not simply the outcome of competing with HPR for a common receptor and/or signaling system.

Overall, these results raise the question of how the two retinoids, RA and HPR, elicit different cellular effects. There have been various suggestions that agents that induce cellular differentiation stimulate the expression of an anti-apoptotic or survival activity to ensure cell viability during cell cycle arrest. It would be expected that such an agent would be selectively
expressed and/or activated by RA and not by HPR. One interesting candidate for such a protective factor is the TGase, given that increased TGase activation is an outcome of RA treatment (6–9). Fig. 4A shows that following the addition of RA to HL60 cells, there is a progressive increase in the TGase protein levels (lower left panel) as well as a corresponding stimulation of GTP binding activity as determined from the incorporation of [γ-32P]GTP (upper panel). However, as pointed out below, RA is able to stimulate the activation of the TGase in HL60 cells through a mechanism that is distinct from simply increasing TGase expression. Both maximal TGase expression and GTP binding activity occurred 2–3 days after RA treatment. A similar experiment in which NIH3T3 cells were treated with RA yielded comparable increases in TGase expression and GTP binding activity (Fig. 4B), whereas the addition of RA to HeLa cells had only modest effects on TGase expression but markedly stimulated its GTP binding, GTP hydrolytic, and transamidation activities (20).

The effects of HPR stimulation on HL60 cells were very different from those of RA. The lower right panel in Fig. 4A
shows the results of an experiment where a 10-fold greater amount of total cellular protein was examined relative to the lower left panel so that it was possible to detect the basal levels of TGase expression in cells (i.e. in the absence of RA treatment). We see that upon treatment of HL60 cells with HPR there is a steady decline in the levels of TGase, such that ultimately no TGase protein is detected. The HPR effects occur within a shorter time period compared with those of RA and are consistent with the time course for HPR-induced apoptosis. Under these conditions the TGase shows no ability to incorporate [\(\gamma^{32}\)P]GTP (Fig. 4A, upper panel). This is even the case prior to the addition of HPR. Thus, despite a detectable level of expression, the TGase apparently exists in an inactive state in the absence of RA treatment, similar to what we had earlier observed in HeLa cells (20).

When HL60 cells were first treated with RA for 2 days and then exposed to HPR, the TGase protein levels were increased and were comparable with those observed when cells were treated with RA in the absence of HPR (Fig. 5A). Treatment with RA prior to HPR addition also yielded a fully active TGase, as indicated by the incorporation of [\(\gamma^{32}\)P]GTP (Fig. 5B). It should be noted that the HPR-induced reduction in TGase levels appears to be due to effects on TGase expression rather than the result of an HPR-mediated proteolytic degradation of the TGase, as indicated by our finding that the histone deacetylase inhibitor can reverse the effects of HPR on TGase expression (data not shown). Thus, pretreatment of cells with RA ensures proper TGase expression and activation, prior to any inhibitory effects on TGase expression that accompany HPR treatment.

The selective expression and activation of the TGase by RA versus HPR caused us to consider the possibility that the TGase represents a protective factor against apoptosis. This led us to examine the consequences of using the TGase inhibitor MDC (32, 33). We determined that treatment of HL60 cells with 100 \(\mu\)M MDC eliminated any protective effects afforded by RA against HPR-mediated apoptosis and in fact caused a marked enhancement in the overall apoptotic response (Fig. 6). Moreover, we found that treatment with MDC converted RA into a potent apoptotic factor. These results suggest that RA is capable of inducing an apoptotic program, perhaps much like HPR, but that the expression and activation of the TGase affords a protective effect. Elimination of TGase activity then allows the apoptotic program to dominate.

Because our data strongly imply that augmentation of TGase expression and activation by RA are required for protection from HPR-induced cell death, we then asked whether simply expressing exogenous TGase in cells (without adding RA) would be sufficient to provide a protective effect from HPR-induced apoptosis. Because HL60 cells are difficult to transfect, we decided to perform this series of experiments in NIH3T3 cells. These mouse fibroblasts are a reasonable alternative for HL60 cells because they are sensitive to HPR-mediated cell death (Fig. 1A), and RA selectively up-regulates TGase expression and activation (Fig. 4B). We initially confirmed that the exogenously expressed TGase in NIH3T3 cells was fully functional as indicated by its ability to incorporate [\(\gamma^{32}\)P]GTP (Fig. 7A, WT TG) and catalyze transamidation, as measured by the incorporation of [\(\beta^{32}\)H]putrescine onto N,N-dimethylcasein (Fig. 7B, WT TG). It is worth noting that the nucleotide binding and enzymatic activities associated with the overexpressed TGase occur without the addition of the stimulatory factor RA. We speculate that this is a result of saturating the negative regulator(s) of TGase activities that has been proposed to function in cells (20). Compared with the TGase-expressing cells, control cells incubated with HPR were twice as sensitive to HPR-mediated cell death (Fig. 7C). Thus, the enhanced viability of RA-treated cells from HPR-mediated apoptosis can be largely explained by the capacity of RA to modify TGase expression and activation.

Several lines of evidence have implicated the transamidation function as being necessary to produce the cellular effects associated with the TGase (34), whereas the nucleotide binding served primarily to modulate this enzymatic activity (14, 18). Given that our data link TGase activity to a novel role in cells, that of being a survival factor, we decided to investigate which of the activities of the TGase elicited this response. We generated a series of point mutations in TGase (initially described by Iismaa et al., Ref. 35) that would be defective in nucleotide binding, transamidation activity, or both activities and tested the ability to promote cell viability. Mutant forms of the TGase that were defective in GTP binding, namely mutants S171E and G165V (Fig. 7A), were most susceptible to HPR-induced cell death (Fig. 7C). In contrast, those TGase forms that retained the capacity to bind nucleotide, WT TG, C277V, and S171C (Fig. 7A), all exhibited protection form the apoptotic effects of HPR.

Expression of WT TGase in NIH3T3 cells resulted in a 17-fold increase in transamidation activity,
whereas expression of the S171C mutant caused a 13-fold increase in the enzymatic activity, as determined by the incorporation of [3H]putrescine onto N,N-dimethylleucine (Fig. 7B). This is particularly interesting because chronic cross-linking activity has frequently been correlated with the induction of apoptosis (34), yet both WT TGase and mutant S171C display elevated transamidation activity and are resistant to the effects of HPR (Fig. 7C). Moreover, TGase transamidation activity appears not to be essential for the prosurvival function of the molecule, because the TGase mutant C277V, which can bind GTP but cannot transamidate (Fig. 7, A and B), can limit the apoptotic response similar to that of WT TG and mutant S171C (Fig. 7C). These findings suggest that chronic transamidation activity does not necessarily have detrimental effects on cells, and that the GTP binding activity of TGase may be sufficient to protect cells from apoptosis under certain conditions.

A developing question in the use of retinoids for cancer therapy is how RA and HPR produce different cellular outcomes given that they are thought to activate a similar pool of receptors and regulate the expression of a common set of genes. We found that one difference between these two retinoids is the selective up-regulation of TGase expression and activation by RA. The changes in protein and activation levels of the TGase appear not to account for the protective effect afforded by RA from HPR-induced apoptosis, but when activity is inhibited can convert RA into a cell death signal. Our results implicating TGase as a survival factor contradict the argument that TGase serves as an apoptotic factor in cells. Increased TGase activity has most often been correlated with the induction of apoptosis (34), yet both WT TGase and mutant S171C display increased transamidation activity and are resistant to the effects of HPR (Fig. 7C). Therefore, TGase activity appears not to be essential for the prosurvival function of the molecule, because the TGase mutant C277V, which can bind GTP but cannot transamidate (Fig. 7, A and B), can limit the apoptotic response similar to that of WT TG and mutant S171C (Fig. 7C). These findings suggest that chronic transamidation activity does not necessarily have detrimental effects on cells, and that the GTP binding activity of TGase may be sufficient to protect cells from apoptosis under certain conditions.

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