Catalase-overexpressing Thymocytes Are Resistant to Glucocorticoid-induced Apoptosis and Exhibit Increased Net Tumor Growth

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ABSTRACT

Glucocorticoids are used for the treatment of lymphoid neoplasms, taking advantage of the well-known ability of these compounds to cause apoptosis in lymphoid tissues. Previously, we have shown that dexamethasone, a synthetic glucocorticoid, causes a down-regulation of several antioxidant defense enzymes and proteins, including catalase and thioredoxin, concomitant with the induction of apoptosis in WEHI7.2 mouse thymoma cells. To test whether this down-regulation plays a critical role in the mechanism of steroid-induced apoptosis, WEHI7.2 cells were transfected with rat catalase. Two clones, expressing 1.4-fold and 2.0-fold higher catalase specific activity, respectively, when compared with vector-only transfectants were selected for further study. An increase to 1.4-fold parental cell catalase activity delayed cell loss after dexamethasone treatment, whereas a 2.0-fold parental catalase activity prevented dexamethasone-induced cell loss for 48 h after treatment. Dexamethasone treatment of the WEHI7.2 cells stimulated a release of cytochrome c into the cytosol. Catalase-overexpressing cells showed a delay or lack of cytochrome c release, suggesting that the mitochondria, which correlated temporally with the delay or prevention of cell loss in the culture after dexamethasone treatment. A decreased amount of cell death from WEHI7.2 cells overexpressing catalase was also seen in tumor xenografts in severe combined immunodeficient mice when compared with tumors from vector-only transfected cells. Similarly, thioredoxin-overexpressing WEHI7.2 cells, shown previously to be apoptosis resistant, showed decreased cell death in tumor xenografts. This resulted in larger tumors from cells overexpressing these proteins. Cell death in control transfected tumor xenografts was primarily attributable to apoptosis. In contrast, the cell death we observed in tumors from thioredoxin- or catalase-overexpressing cells had a higher frequency of a nonapoptotic, nonnecrotic type of cell death termed para-apoptosis. These data suggest that: (a) oxidative stress plays a critical role in steroid-induced apoptosis prior to the commitment of the cells to undergo apoptosis; and (b) resistance to oxidative stress can contribute to tumor growth.

INTRODUCTION

Apoptosis of lymphoid tissues attributable to glucocorticoid treatment is a classic example of apoptosis cited in the early descriptions of apoptotic morphology (1). This process is important physiologically, especially for the sorting of maturing lymphocytes (2). Clinically, this feature of glucocorticoids has been exploited for the treatment of leukemia and lymphoma; unfortunately, as with many chemotherapeutic agents, the development of drug resistance is a problem (3–5). Glucocorticoids exert their action primarily by binding to a cytosolic receptor that translocates to the nucleus and affects transcription (6). Lack of functional receptors desensitizes lymphocytes and thymocytes to glucocorticoid-induced apoptosis both in tissue culture cell lines and animal models (7–11). In some patients, drug resistance can be traced to either a receptor mutation or a decrease in functional glucocorticoid receptor number (3, 5); however, in other individuals resistance cannot be traced to glucocorticoid receptor alterations (12). The events that occur after translocation of the receptor/steroid complex to the nucleus and prior to the commitment of the cell to die are not understood. Elucidation of the mechanism by which glucocorticoids kill lymphocytes may make it possible to target alternative steps in the process for chemotherapeutic intervention, particularly when drug resistance occurs.

The necessity for functional glucocorticoid receptors and RNA synthesis (13, 14) during steroid-induced lymphocyte apoptosis suggests that identifying alterations in mRNA transcripts may provide insight into critical steps required for this process. Previously, we found that treatment of WEHI7.2 mouse thymoma cells with dexamethasone, a synthetic glucocorticoid, causes down-regulation of a number of antioxidant defense enzymes (15, 16). Glutathione peroxidase and catalase, which metabolize cellular H2O2; superoxide dismutase, which detoxifies O2− and thioredoxin, a small protein involved in producing reducing equivalents, are all down-regulated at the mRNA level (16). Concomitant with the antioxidant enzyme down-regulation is an increase in the mRNA for glutathione S-transferase (15), an enzyme thought to be involved in the removal of lipid peroxidation products (17). This suggests a model whereby steroids down-regulate cellular antioxidant defenses, which decreases the ability of the cell to detoxify ROS3 produced via normal metabolism and results in apoptosis.

There is some evidence that ROS may be involved in steroid-induced apoptosis of lymphocytes. Exogenous treatment of thymocytes with chemical antioxidants, antioxidant enzymes, or metal chelators protects against dexamethasone-induced apoptosis (16, 18–23). Culture of thymocytes under hypoxic conditions generally affords protection from dexamethasone (16, 22, 24). Furthermore, lipid peroxidation is seen after glucocorticoid treatment in thymocytes (25) and S49.1 lymphocytes (26), suggesting that oxidative damage occurs during steroid-induced apoptosis in these cells. Finally, WEHI7.2 cells overexpressing thioredoxin are resistant to steroid-induced apoptosis (27).

To determine whether the previously observed antioxidant defense enzyme down-regulation is critical during steroid-induced lymphocyte apoptosis, we have isolated two WEHI7.2 catalase transfectants. These cell variants have provided useful tools to test the importance of catalase to the mechanism of steroid-induced apoptosis in vitro and in vivo while helping to elucidate the role of ROS in this process.

MATERIALS AND METHODS

Cell Culture and Transfection. The mouse thymoma-derived WEHI7.2 parental cell line (28) was obtained from Dr. Roger Miesfeld (University of Arizona). Cells were maintained in DMEM-low glucose (Life Technologies, Inc., Grand Island, NY) supplemented with 10% calf serum (Hyclone Laboratories, Logan, UT) at 37°C in a 5% CO2 humidified environment. Cells were maintained in exponential growth at a density between 0.02 and 2 × 106 cells/ml. Stable transfectants of WEHI7.2 cells overexpressing thioredoxin have been characterized previously and were maintained as described (27).

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3 The abbreviation used is: ROS, reactive oxygen species.
Catalase-overexpressing cells were constructed by stably transfecting WEHI7.2 cells with a vector containing rat catalase cDNA inserted into the KpnI/Ncol sites of the pBK-CMV vector (constructed and kindly donated by Dr. Melissa Williams, University of Texas Health Sciences Center, San Antonio, TX). Cells were transfected, and positive clones were selected as described previously (27). All studies were conducted on clonal lines maintained in 800 μg/ml G418 (Life Technologies, Inc.) and then cultured in medium in the absence of selection drug 1 week prior to each experiment. Immediately before each experiment, live cells were separated from those that underwent spontaneous apoptosis using Ficoll-Plaque Plus reagents and the manufacturer’s suggested protocol (Amersham Pharmacia Biotech, Piscataway, NJ).

Chemicals and Drugs. All chemicals and drugs used in these experiments were obtained from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted.

Glucocorticoid Receptor Number. The number of functional glucocorticoid receptors/cell was determined by using a transient cotransfection of cells with a glucocorticoid response element/chloramphenicol acetyl transferase reporter plasmid and a β-galactosidase expression vector as described previously (27).

Sensitivity to Dexamethasone. Sensitivity to dexamethasone-induced apoptosis in cell culture was measured by treating cells with either 1 μM dexamethasone or an equivalent volume of ethanol vehicle alone (final ethanol concentration, 0.01%). Periodically, an aliquot of cells was removed and resuspended with an equal volume of an Eosin Y solution (500 μg/ml final dye concentration), allowed to stand 2–3 min at room temperature, and counted using a hemocytometer. Cells that excluded dye were scored as viable. To confirm that cell loss in culture after dexamethasone treatment was attributable to apoptosis, an aliquot of cells was prepared for morphological examination as described under microscopy.

Enzyme Activity Assay. Prior to enzyme activity measurements, cells were washed twice at 4°C with PBS, 137 mM NaCl, 2.7 mM KCl, 8.1 mM NaHPO4, and 1.5 mM KH2PO4, pH 7.4. Cells were resuspended in 10 mM Tris-HCl (pH 7.5), 250 mM sucrose, 1 mM EDTA, 0.5 mM DTT, 0.1 mM phenylmethylsulfonyl fluoride, and 1% Triton X-100. Samples were incubated for 30 min on ice and centrifuged at 10,000 × g for 15 min at 4°C. Activity was measured in the supernatant fractions as the decrease in H2O2 absorbance at 240 nm as described by Beers and Sizer (29) and calculated using an extinction coefficient of 43.6 M–1 cm–1. Cellular protein was measured using the BCA protein assay kit (Pierce, Rockford, IL) according to the manufacturer’s protocol, and all enzyme activities were normalized to cellular protein.

In Vivo Tumor Growth. Tumor formation in vector-only, thioridoxin-transfected, and catalase-transfected WEHI7.2 cells was compared by injecting 2 × 103 cells in a 0.2-ml Matrigel:0.9% NaCl (50:50) mixture, s.c., into the flanks of female severe combined immunodeficient mice. Catalase and vector-only transfecants were injected into 20 mice, whereas 18 received thioridoxin-transfected cells. Tumor volume was measured with calipers every 3 days. On the tenth day after tumor cell injection, daily injections of 1 mg/kg body weight dexamethasone in a 10% ethanol:0.9% NaCl vehicle were begun in half the mice from each cell variant group. Control mice were injected with vehicle alone. Seventeen days after tumor cell injection, four mice from each cell variant group receiving dexamethasone and four receiving vehicle alone were euthanized, the tumors were excised, and a portion of the tumor was fixed for microscopy. On day 20 after tumor cell injection, the remaining animals were euthanized, and tumors from three mice in each category were weighed.

Microscopy. Portions of the tumors excised on day 17 were fixed in one-half strength Karnovsky’s fixative overnight, postfixed in osmium tetroxide, dehydrated in a graded series of alcohols, and embedded in epoxy resin. One μm-thick sections were prepared and stained with toluidine blue for light microscopy. Cells were scored as apoptotic, mitotic, normal, or para-apoptotic using a light microscope. Then the membranes were washed once for 15 min and five times for 5 min each with PBS-T. Horseradish peroxidase-linked antirabbit immunoglobulin antibody (Amersham Pharmacia Biotech) was diluted 1:10,000 in 1% BSA/PBS-T and incubated with the membranes for 1 h. Then the membranes were washed once for 15 min and five times for 5 min each with PBS-T. Proteins were detected by chemiluminescence using the manufacturer’s protocol (ECL Western Blotting System; Amersham Pharmacia Biotech) and film (X-OMAT Blue; Eastman Kodak, Rochester, NY).

Measurement of Cytochrome c. Cell cultures were treated with 1 μM dexamethasone or vehicle as described above and harvested by centrifugation (1000 × g for 5 min at 4°C). Cell pellets were washed with PBS, and the cytosolic (S100) and mitochondrial fractions were prepared according to Vander Heiden et al. (33), except that cells were resuspended in buffer A without sucrose and allowed to sit for 30 min before the addition of sucrose to make a final concentration of 250 mM. Cells were immediately lysed using a glass tissue homogenizer with a glass pestle (Kontes, Vineland, NJ). Proteins from cytosolic and mitochondrial fractions (30 μg of each sample) were separated on a 15% SDS-PAGE gel (31), and proteins were transferred as described above. Blots were probed for cytochrome c using 1 μg/ml anti-cytochrome c antibody (PharMingen, San Diego, CA) and cytochrome c oxidase with 1 μg/ml anti-cytochrome oxidase subunit I antibody (Molecular Probes, Eugene, OR), using the manufacturer’s suggested protocol accompanying the cytochrome c antibody. Proteins were detected by first incubating with a 1:2000 dilution of horseradish peroxidase-linked antirabbit immunoglobulin (Amersham Pharmacia Biotech) and detecting the product by chemiluminescence (Renaissance Western Blot Chemiluminescence Reagent Plus; NEN Life Science Products), as suggested by the manufacturer.

Statistics. The tumor growth rate was compared by first fitting a least square regression line to the cube root of tumor volume by day for each mouse. The line slopes were then compared in a two-way ANOVA to test the difference in tumor growth rate between categories. This analysis was performed by the Statistics Core at the Arizona Cancer Center. The rest of the data were compared using Student’s t test assuming unequal variances. Significance was set at P < 0.05.

RESULTS

To verify the increased expression of catalase in the transfectants, catalase activity was measured, and two clones were selected for further study. Catalase activity in the CAT38 clone was ~1.4-fold that seen in the vector-only transfectants (Neo2 and Neo3), whereas the CAT2 clone exhibited 2.0-fold greater catalase activity (Fig. 1B).
Different from triplicate measurements in an experiment that has been replicated; bars, significantly different from vector-only (A shown). On the basis of [3 H]dexamethasone binding assays, WEHI7.2 cultures were used for comparison. As expected, tumors from thioredoxin-transfected WEHI7.2 cells were used as a positive control (Lane STD). We were unable to select clones expressing enough catalase to cause a significant phenotype.

Addition of antioxidants to the culture medium protects thymocytes from glucocorticoid-induced apoptosis (16), suggesting that ROS are involved in this cell death. To test whether the increased cellular catalase protects cells from steroid-induced apoptosis, the viable cell number over time after the addition of 1 μM dexamethasone in catalase transfectant cell cultures was compared with that in cultures with vector-only transfected cells (Fig. 2). The CAT38 cells showed a delayed cell loss when compared with the vector-only transfected cells. After a 48-h treatment with dexamethasone, a significantly greater number of viable cells remained in the CAT38 cultures than in either of the two neo-transfectant cultures. CAT2 cells, which have the higher catalase activity, had a decreased cell loss when compared with that in the CAT38 cell cultures. CAT2 cell cultures contained significantly more viable cells than either CAT38 or neo-transfectant cultures 48 h after dexamethasone treatment. In the absence of dexamethasone, cells grew exponentially with similar growth rates (data not shown). Morphological assessment of cells pelleted from these cultures verified that cell loss was attributable to apoptosis (data not shown). The pattern of cytotoxicity seen in Fig. 3 was not attributable to contamination of the cytosolic preparation with mitochondria because the cytochrome oxidase subunit I (a mitochondrial specific protein) content of all cytosolic preparations was minimal and similar (data not shown).

Apparent tumor growth rate depends on both the rates of cell growth (as measured by mitosis) and cell loss (often by apoptosis). To test whether altering catalase affected tumor growth and response to dexamethasone, we compared tumor volume and weight in mouse tumor xenografts from vector-only transfected, catalase-transfected, and thioredoxin-transfected WEHI7.2 cells (Fig. 4, A and B). The CAT38 cells were chosen for this experiment because the catalase activity remains elevated in the absence of selection drug for a sufficient time period for this type of study (data not shown). Thioredoxin-transfected WEHI7.2 cells were used as a positive control because it has been shown previously that increased thioredoxin causes increased net tumor growth (27). Twenty days after cell injection, average tumor weight from both catalase and thioredoxin transfected tumor xenografts was significantly greater than that from Neo3 tumor xenografts (Fig. 4B). Tumor volume measurements on a day-to-day basis were somewhat variable; therefore, a growth rate for each tumor was calculated, and these values were used for comparison. As expected, tumors from thioredoxin-transf-
fected cells showed a significantly increased tumor growth rate when compared with that from the vector only cells (Neo3; Fig. 4C). Tumor xenografts from cells with increased catalase also appeared to grow slightly faster than the Neo3 cell tumors, although this difference was not significant. Unexpectedly, no significant effect of dexamethasone on tumor weight or tumor growth rate was seen. This lack of dexamethasone response may be because the dose of dexamethasone was inadequate or the timing of the doses made it difficult to measure an effect.

Alterations in tumor weight appeared to be primarily attributable to alterations in spontaneous cell death (Fig. 5A). Total cell death was significantly greater in Neo3 tumors when compared with tumors from thioredoxin- or catalase-transfected cells (Fig. 5B). Mitosis was similar in sections of tumors from all three cell variants (data not shown). Most of the cell death observed in the sections from the Neo3 tumors was apoptosis (Fig. 6A, top). In tumors from the thioredoxin- or catalase-transfected cells, an increased amount of cell death, morphologically distinct from apoptosis, was observed (Fig. 6A, bottom, and B). The classic ultrastructural features of apoptosis, including margination of chromatin, nucleolar exclusion, and cell shrinkage as shown in Fig. 7A, were absent from a large portion of the dead cells from thioredoxin or catalase transfectant tumor xenografts. Instead, these dead cells exhibited a type of cell death termed para-apoptosis (39, 40). The para-apoptotic cells had very extensive processes that squeezed between adjacent cells (Fig. 7B) and displayed a network of cytosolic fibers (Fig. 7C), neither of which is a characteristic of apoptotic cells. In contrast to the electron lucency of necrotic cells, both cytoplasm and nucleus of the para-apoptotic cells were very electron dense. The mitochondria were also distended and showed a

Fig. 3. Comparison of cytochrome c release into the cytosol in WEHI7.2 parental cells, vector-only transfectants (Neo3) and catalase-transfected (CAT) cells. Representative immunoblots of the cytosolic (S100) and mitochondrial (MT) cellular fractions probed with anti-cytochrome c primary antibody show the relative cytochrome c content. Lane E, vehicle-only treated control; Lanes 8D–40D, time (8–40 h) in dexamethasone (D). Lane EMT contains 10 μg of control cell mitochondrial fraction protein used as a positive control.

Fig. 4. Growth in tumor xenografts from cells transfected with catalase, thioredoxin, or vector only. A. Comparison of representative tumors from vector-only (Neo3, NEO), catalase (CAT38, CAT), or thioredoxin (THX6, THX) transfected WEHI7.2 cells 17 days after cell injection. B. Average weight of tumor xenografts 20 days after cell injection (n = 6); bars, SE. *, significantly different from Neo3 values. C. Mean tumor volume (days 0–17, n = 8; day 20, n = 4) in tumor xenografts from Neo3, CAT38, or THX6 cells; bars, SE. The daily injections, 1 mg/kg dexamethasone (D) or vehicle only (E), were started on day 10.
loss of cristae, features not observed in apoptosis. These para-apoptotic cells appeared very similar to those described as “type B dark cells” (41, 42).

**DISCUSSION**

Catalase overexpression protects thymocytes from glucocorticoid-induced apoptosis. The degree of protection is proportional to the level of catalase overexpression such that the higher the catalase activity the greater the protection. The normal cellular function of catalase is to detoxify H$_2$O$_2$, resulting in the formation of H$_2$O and O$_2$ (43). Although catalase is a peroxisomal enzyme in most cells, H$_2$O$_2$ is thought to be readily diffusible; therefore, overexpression of catalase overexpression results in increased tolerance to glucocorticoid-induced apoptosis. This phenomenon is not unique to cancer cells and has been observed in normal cells as well (44).
lase, even in the peroxisomes, should result in decreased intracellular 
\( \text{H}_2\text{O}_2 \) (43–45). Our data suggest that 
\( \text{H}_2\text{O}_2 \) plays a critical role in 
glucocorticoid-induced thymocyte apoptosis.

The protection provided by the modest increases in catalase activity 
seen here suggests that even small changes in ROS may be critical. 
Although ROS measurements using whole-cell populations have sug-
gested that little, if any, increase in ROS occurs (25, 26, 46), separa-
tion of the apoptotic populations by flow cytometry (23) or Percoll 
gradients (20) have shown that small increases in ROS occur in the 
apoptotic cells after dexamethasone treatment. Indications that ROS 
might play a critical role come from studies that show that treatment 
of thymocytes in culture with antioxidant enzymes or chemical anti-
oxidants is protective (16, 18–23). Hypoxic conditions, where lower 
ROS production is expected, are also generally protective (16, 22, 24).

Treatment with divalent metal ion chelators protects thymocytes in 
culture, suggesting a role for Fenton chemistry in the mechanism of 
steroid-induced killing (18). A decrease in reduced glutathione (15, 
20, 21, 25) and the appearance of lipid peroxidation products (25, 26) 
are also observed after steroid treatment, suggesting that cells are 
undergoing oxidative stress during glucocorticoid-induced apoptosis.

Dexamethasone induces apoptosis in the WEHI7.2 cells by stimu-
lating the release of cytochrome \( \text{c} \) from the mitochondria into the 
cytosol. In this apoptotic pathway, some as yet unidentified series of 
events (the signal) impinges on the mitochondria to stimulate the 
release of cytochrome \( \text{c} \) into the cytosol. Release of cytochrome \( \text{c} \), 
considered the committed step, then triggers the activation of apical 
caspases. Apical caspases activate effector caspases, which in turn 
degrade cellular substrates in what is considered the execution phase 
(38). Cytochrome \( \text{c} \) release is also thought to trigger the hyperpro-
duction of ROS common to the later stages of apoptosis induced by 
many agents (21, 47, 48).

Overexpression of catalase delays or prevents the release of cyto-
chrome \( \text{c} \) into the cytosol, suggesting that ROS, in particular \( \text{H}_2\text{O}_2 \), 
acts in the signaling phase of glucocorticoid-induced lymphocyte 
apoptosis. This protection occurs before the committed step and is 
therefore separable from late-stage ROS hyperproduction. Other stud-
ies that have attempted to determine the sequence of apoptotic events 
have suggested that ROS increases may be an early event in steroid-
duced apoptosis. The small increases in ROS seen in two studies 
(23, 25) and the lipid peroxidation seen by Bustamante et al. (25) 
appeared shortly after steroid treatment. Work by Macho et al. (21) 
and Zamzami et al. (49) has also shown that addition of catalase or 
\( \text{N} \)-acetylcysteine, an antioxidant and glutathione precursor, to lympho-
cyte cultures delays the irreversible loss of mitochondrial trans-
membrane potential and the hyperproduction of ROS. To act as 
biological signals, molecules need only brief appearances at low 
concentrations to be effective. This may explain how modest increases 
in ROS play a critical role during steroid-mediated apoptosis. A 
number of the signaling pathways known to be redox sensitive or 
affected by ROS directly (50, 51) are involved in apoptosis. Our data 
suggest that a closer look at redox-sensitive signaling pathways may 
help determine the mechanism of steroid-induced apoptosis.

Tumor cell resistance to the chemotherapeutic effects of glucocor-
ticoids may be connected to increased resistance to ROS. Loss of 
fungal glucocorticoid receptors is a cause of resistance in some 
patients (3, 5); however, resistance in other individuals cannot be 
traced to glucocorticoid receptor alterations (12). Overexpression of 
catalase (as shown here) or thioredoxin (27), both part of the cellular 
antioxidant defense, protects thymocytes from glucocorticoid-induced 
apoptosis in vitro without alterations in functional receptors. Simi-
larly, WEHI7.2 cells selected for resistance to \( \text{H}_2\text{O}_2 \) show a bolstered
antioxidant defense and resistance to steroid-induced apoptosis. The dexamethasone-resistant WEHI7.2 cell mutants isolated by Flomerfelt and Miesfeld (52) also show cross-resistance to H2O2. Although no dexamethasone effect was seen in vivo in the present study (possibly because of experimental design parameters), these data suggest that antioxidant status may help predict the efficacy of glucocorticoid chemotherapy when receptors are normal.

Constitutive catalase or thioredoxin overexpression in WEHI7.2 cells causes net increased tumor growth by decreasing apoptosis in the tumors. Both thioredoxin and catalase have been shown to protect against apoptosis. Thioredoxin added to the culture medium or overexpressed protects cells from apoptosis induced by diverse agents such as thiol depletion, tumor necrosis factor, and staurosporine, some of which are thought to kill cells via ROS production (27, 53–56). Recently, it has been shown that thioredoxin can inhibit apoptosis by inhibiting the apoptosis signal-regulating kinase-1 in a redox-sensitive manner (50, 57), suggesting that thioredoxin could inhibit any apoptotic pathway that uses this molecule. Addition of catalase to the culture medium or overexpression of catalase has also been shown to protect cells from apoptosis and oxidative stress (examples in Refs. 21, 44, 53, and 58–61, among many others). Although antioxidants are generally considered anticarcinogenic and tumor cells often show decreased antioxidant enzyme expression (62, 63), increased antioxidant enzyme expression in tumor tissue has also been reported. Increased catalase has been reported in some tumor tissue in the thyroid (64) and larynx (65) when compared with noncancerous controls. Elevated thioredoxin has been reported in a portion of lung and colon tumors (66, 67) as well as neoplastic cervical squamous epithelium and hepatocellular carcinoma (68, 69). As shown here and previously for thioredoxin (27), constitutive overexpression of an antioxidant enzyme or protein in WEHI7.2 cells enhances tumor growth. Constitutive overexpression of glutathione peroxidase causes enhanced skin carcinogenesis in mice using the 7,12-dimethylbenz[a]anthracene/12-O-tetradecanoylphorbol-13-acetate, two-stage skin carcinogenesis mouse model (70). Maintenance of antioxidant enzyme activities within a specific range may be the important factor for prevention of tumor growth. Inhibition of cell death may be a key factor in the apparent tumor growth observed with antioxidant enzyme overexpression or in tumors with elevated antioxidant enzyme activity.

Antioxidant enzyme overexpression may also affect apparent tumor growth by affecting tumor cell removal, as suggested by Lu et al. (70), to explain the enhanced tumor growth seen with glutathione peroxidase overexpression. Increases in antioxidant enzymes may render cells more resistant to killing by the oxidative burst delivered by cells of the immune system. In addition, H2O2 increases MHC class I expression (71). Overexpression of antioxidant enzymes may effectively scavenge H2O2, resulting in a decreased MHC surface expression in these cells and therefore a decreased removal by macrophages. Although overall cell death was decreased in tumors from catalase and thioredoxin-overexpressing cells, there was a significant increase in the number of cells that showed para-apoptotic morphology (also referred to as type B dark cell death; Refs. 41 and 42). Cells with this type of morphology are generally considered dead because the mitochondria appear swollen with fragmented cristae (72). This type of cell death has been described in both physiological (73) and pathological (72, 74–76) conditions. Although morphologically characterized, little is known about the cause or the biochemical pathways involved in this type of death. The best characterized are the mammified Hodgkin and Reed-Sternberg cells of Hodgkin’s disease. Hodgkin and Reed-Sternberg cells that exhibit this morphology do not show the DNA fragmentation characteristic of apoptosis or alterations in p53 or Bcl-2 (75, 77). The increased incidence of this type of cell death in tumor xenografts from antioxidant enzyme-overexpressing cells suggests that cellular redox status may play a role in para-apoptosis.

These data lend support to a critical role for antioxidant enzymes in tumorigenesis. The cell variants developed here will provide useful tools to investigate antioxidant enzyme status as a predictor of chemotherapeutic response or target for intervention.

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