Induction of Mr 78,000 Glucose-regulated Stress Protein in Poly(Adenosine Diphosphate-Ribose) Polymerase- and Nicotinamide Adenine Dinucleotide-deficient V79 Cell Lines and Its Relation to Resistance to the Topoisomerase II Inhibitor Etoposide

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ABSTRACT

Cell lines deficient in poly(ADP-ribose) synthesis due to enzyme deficiency (ADPRT54 and ADPRT351) or substrate deficiency (N2, N3, and N4) are resistant to topoisomerase II-directed agents, including etoposide (VP-16), N-[4-(9-acridinylamino)-3-methoxyphenyl]methanesulfonamide, and Adriamycin, relative to the effect of these agents on parental V79 Chinese hamster cells. Resistance is stable in the ADPRT54 and ADPRT351 cell lines, whereas resistance in the N2, N3, and N4 cell lines occurs when the cells are grown in nicotinamide-deficient medium to produce a state of NAD deficiency. However, sensitivity to VP-16 reverts to normal when cellular NAD levels return to control levels during growth in nicotinamide-containing complete medium. Poly(ADP-ribose) polymerase-deficient cell lines show constitutively increased levels of a protein at Mr 78,000 on Coomassie blue-stained, sodium dodecyl sulfate-polyacrylamide gels that was subsequently confirmed with monoclonal antibodies to be Mr 78,000 glucose-regulated stress protein (GRP78). Similarly, N2, N3, and N4 cell lines show induction of GRP78 under nicotinamide-deficient conditions. Induction of GRP78 is associated with elevated levels of GRP78 mRNA and appears to be regulated at the transcriptional level. When N3 cells with deficiency of poly(ADP-ribose) synthesis due to NAD deficiency are shifted to complete, nicotinamide-containing medium, they restore their NAD content, undergo a decrease in GRP78 levels, and regain sensitivity to VP-16. When V79 cells are shifted to nicotinamide-deficient medium they undergo a reduction in NAD content, followed by a progressive elevation in GRP78 levels, and they subsequently become increasingly resistant to VP-16. These studies demonstrate a clear association between deficiency of the NAD-poly(ADP-ribose) synthesis system, induction of GRP78 synthesis, and resistance to VP-16.

INTRODUCTION

Poly(ADP-ribose) polymerase is a chromatin-bound enzyme that utilizes cellular NAD for the synthesis of ADP-ribose homopolymers attached to several nucleic proteins, including nuclear matrix proteins, histones, topoisomerase II, and the enzyme poly(ADP-ribose) polymerase itself (1–3). Poly(ADP-ribose) polymerase is clearly activated by DNA strand breaks and its activity has been implicated in the response to DNA damage as well as in processes of DNA replication, repair, transcription, and differentiation (2–4). To better understand the role of poly(ADP-riboseylation) in cellular processes, we developed two types of mutant cell lines that are deficient in their ability to synthesize poly(ADP-ribose) (5, 6). ADPRT54 and ADPRT351 cell lines were derived from Chinese hamster V79 cells by a strategy designed to select cell lines deficient in the enzyme poly(ADP-ribose) polymerase. These cell lines have <5–11% poly(ADP-ribose) polymerase under conditions of maximal enzyme stimulation. N2, N3, and N4 cell lines were derived from V79 cells by a strategy designed to select cell lines capable of persistent growth in the absence of nicotinamide or any of its analogues in the growth medium. When grown in nicotinamide-deficient medium, these cell lines have NAD levels of 1–3%, relative to their parental V79 cells. Thus, they have an impaired ability to synthesize poly(ADP-ribose) because of their substrate deficiency. When nicotinamide-deficient growth medium is replaced with nicotinamide-containing medium, NAD levels become normal within hours and under these conditions N2, N3, and N4 cells regain their capacity for normal poly(ADP-ribose) synthesis (5, 6).

We have previously shown that ADPRT54, ADPRT351, N2, N3, and N4 are hypersensitive to alkylating agents, topoisomerase II inhibitors, and UV- and X-irradiation (7–9). In contrast, these cell lines are highly resistant to topo II-targeted drugs such as VP-16, N-[4-(9-acridinylamino)-3-methoxyphenyl]methanesulfonamide, and Adriamycin (8, 10). Topo II-active drugs such as VP-16 initiate their cytotoxic effects by stabilizing a topo II-DNA complex, thus preventing the rapid turnover of topo II-mediated protein-cross-linked-DNA strand breaks and interfering with alterations in DNA topology required for processes such as DNA replication, repair, and transcription (11–14). Prolonged stabilization of topo II-DNA complexes probably leads to DNA recombination events, resulting in genetic rearrangements and deletions which subsequently cause cell death (15–18). While poly(ADP-ribose) polymerase and topoisomerase II are both nuclear enzymes and both are involved in processes of DNA replication, repair, and transcription, it is not apparent why cells deficient in poly(ADP-ribose) synthesis should be resistant to topoisomerase II inhibitors.

Previous studies have shown that treatment of cells with agents or conditions capable of inducing GRP, such as calcium ionophore, 2-deoxyglucose, anoxia, or glucose deprivation, all confer resistance to topo II-targeted drugs (19, 20). Removal of each GRP-inducing condition results in the rapid disappearance of this resistance in a manner that correlates with the repression of the GRPs (19, 20). These observations, along with the fact that poly(ADP-ribose) polymerase and its substrate NAD function as a stress-responsive system following induction of DNA strand breaks, prompted us to determine whether a deficiency of either poly(ADP-ribose) polymerase or NAD is associated with the induction of stress-responsive proteins and resistance to topo II inhibitors. In this manuscript we show that the deficiency of poly(ADP-ribose) synthesis is associated with the induction of GRP78 and subsequent development of resistance to VP-16.

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The abbreviations used are: topo II, topoisomerase II; VP-16, etoposide (Vepesid); GRP, glucose-regulated stress protein; GRP78, glucose-regulated stress protein of Mr 78,000; cDNA, complementary DNA; SDS, sodium dodecyl sulfate.
MATERIALS AND METHODS

Materials. VP-16, a gift from Bristol-Myers Squibb Co. (Evansville, IN), was dissolved and used as described previously (10, 12). Acrylamide and other chemicals for gel electrophoresis were from Bio-Rad (Hercules, CA). The enhanced chemiluminescence kit and horseradish peroxidase-conjugated anti-rat IgG were from Amersham (Arlington Heights, IL). [α-32P]-dCTP (6000 Ci/mmol) was from DuPont-New England Nuclear (Boston, MA). The random-primed DNA labeling kit was from Boehringer Mannheim (Indianapolis, IN). Immobilon-P membranes for Western blotting were from Millipore Corp. (Bedford, MA). Rat monoclonal antibody against GRP78 was developed by Dr. David Bole, University of Michigan (Ann Arbor, MI), and obtained from Dr. Clark Distelhorst, Case Western Reserve University School of Medicine (Cleveland, Ohio) (21).

Cell Lines. The isolation and characterization of all cell lines has been published previously, and their characteristics have been stable for years (6). ADPRT54 and ADPRT351 are cloned cell lines derived from V79 Chinese hamster cells; they have 5–11% poly(ADP-ribose) polymerase activity, relative to their parental cells (6). N2, N3, and N4 are cloned cell lines derived from V79 cells selected for their ability to stably proliferate in the absence of free nicotinamide or any of its analogues. When these latter cell lines are grown in the absence of nicotinamide, they maintain NAD levels in the range of 1–3% of that found in their parental V79 cells grown in complete medium. When the nicotinamide-deficient growth medium is replaced with complete medium, the cells resume normal pyridine nucleotide synthesis and restore their NAD levels to the range of V79 cells within 6 h (6). Since these cell lines are all similar, we describe the results obtained with any one of these cell lines for a particular experiment.

Cell Culture Maintenance and Clonogenic Survival Assays. V79 cells and derivatives were grown in α-modified Eagle’s medium buffered to pH 7.2 with 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and supplemented with 100 units/ml penicillin, 100 μg/ml streptomycin, and 5% heat-inactivated fetal calf serum (6). For growth under nicotinamide-deficient conditions, cells were maintained in the same medium prepared without nicotinamide (K.C. Biologicals, Lenexa, KS) and supplemented with 5% extensively dialyzed, heat-inactivated fetal calf serum. Procedures for determining clonogenic survival were described previously (7–10), except that after VP-16 treatment NAD-deficient cells were grown in regular, nicotinamide-containing medium for colony formation, to avoid any artifact that might have resulted from their growth in NAD-deficient medium during colony formation. Cellular NAD content was measured by enzymatic cycling techniques as described previously (5, 6).

Cytosol Preparation, Gel Electrophoresis, and Western Blotting. Cytosol preparations were done according to previously published methods (22). Cells were collected by centrifugation and lysed by resuspension and incubation for 15 min at 4°C in buffer containing 50 mM Tris-Cl, pH 7.4, 150 mM NaCl, 10 mM glucose, 10 mM iodoacetamide, and 1% Triton X-100. The sample was then centrifuged at 14,000 × g for 20 min at 4°C and the clear supernatant cytosol was collected. SDS-polyacrylamide gel electrophoresis was according to the method of Laemmli (23). Protein was determined according to the method of Bradford (24), using a Bio-Rad protein assay kit (Bio-Rad). Fifty-μg cytosolic protein samples were separated by electrophoresis on a 7.5% polyacrylamide gel and analyzed after Coomassie blue staining. For Western blotting, 10 μg of cytosolic protein samples were separated by electrophoresis on a 7.5% polyacrylamide gel, followed by electrophoretic transfer onto Immobilon-P membrane. The membrane was then incubated overnight at 4°C with a 1:200 dilution of rat anti-GRP78 monoclonal antibody, followed by reaction with a horseradish peroxidase-conjugated second antibody and immunodetection by a chemiluminescent assay employing the enhanced chemiluminescence Western blotting detection system (Amersham). Chemiluminescence was recorded by exposing the membrane to Kodak XAR-5 film at room temperature for appropriate periods of time.

Northern Blotting. Total cellular RNA was isolated by RNA-STAT 60 (Tel-Test “B,” Inc., Friendswood, TX). Samples containing 10 μg of RNA were electrophoresed on a 1% agarose gel and blotted onto Zeta-Probe membranes (Bio-Rad). Membranes were simultaneously hybridized with a cDNA (p35S) (developed by Dr. Amy Lee, University of Southern California) containing the coding sequence for hamster GRP78 (25) and a cDNA corresponding to CHO-B, a constitutively expressed mRNA, given by Dr. Clark Distelhorst, Case Western Reserve University School of Medicine (Cleveland OH). cDNAs were labeled with [α-32P]-dCTP using a random-primed DNA labeling kit. The standard Bio-Rad protocol was followed for prehybridization, hybridization, and washing of the membranes. Finally, the membranes were exposed to Kodak XAR-5 film at −80°C for appropriate periods of time and the bands were quantitated by densitometric scanning.

RESULTS

Cytotoxicity of VP-16 in Poly(ADP-ribose) Synthesis-deficient and -proficient Cell Lines. Fig. 1 shows the clonalogenic survival of the different V79 derivative cell lines following 1-h treatment with increasing concentrations of VP-16. ADPRT54 and ADPRT351 cell lines, which are impaired in poly(ADP-ribose) synthesis because of a deficiency in the enzyme, are clearly resistant to VP-16, compared to their parental V79 cells. N3 cells, which are impaired in poly(ADP-ribose) synthesis due to deficiency in the enzyme substrate NAD, are also resistant to VP-16, relative to their parental V79 cells. When N3 cells are grown in nicotinamide-containing medium for extended periods, their sensitivity to VP-16 is restored almost to that of the parental V79 cells. Thus, cells deficient in their ability to synthesize poly(ADP-ribose) based on enzyme or substrate deficiency are clearly resistant to VP-16-induced cytotoxicity. However, in the case of the substrate-deficient cells, restoration of NAD to normal levels reverses VP-16 resistance.

Effects of Restoration of NAD Levels in NAD-deficient Cells on the Cytotoxicity of VP-16. In subsequent experiments we shifted N4 cells from nicotinamide-deficient medium to nicotinamide-containing regular medium to allow restoration of NAD levels. At daily intervals after shifting to regular medium, we examined the NAD content of the cells and their sensitivity to VP-16 using clonogenic survival assays, VP-16 RESISTANCE IN POLY(ADP-RIBOSE)-DEFICIENT CELLS
to determine the relationship between restoration of NAD levels and disappearance of VP-16 resistance. Fig. 2A shows that the NAD level in N4 cells increased from 230 pmol/mg protein to 4300 pmol/mg protein within 1 day of shifting to regular medium, after which elevated levels were maintained for the next 3 days. Fig. 2B shows that the N4 cells progressively regained their sensitivity to VP-16 during continued growth in regular medium. These results clearly indicate that deficiency of NAD in N4 cells is associated with resistance to VP-16. Furthermore, the results show that the rapid increase in NAD levels that occurs within 24 h is associated with a more gradual increase in VP-16 sensitivity that occurs over a period of 4 days.

To explain these results we suggest that deficiency of NAD results in the induction of a protein that contributes to the development of resistance to VP-16. Upon growth in regular medium, the NAD level is rapidly restored and consequently the induction process is stopped. Subsequently, with continued growth of the cells in regular medium the induced protein gradually decays, resulting in progressive restoration of VP-16 sensitivity.

Protein Induction in Poly(ADP-ribose) Synthesis-deficient Cell Lines. Since poly(ADP-ribose) polymerase and NAD function as a stress-responsive system activated by DNA strand breaks, we examined the effect of the absence of this system on the induction of stress proteins. As shown in Fig. 3A, the cytosolic fractions of the different cell lines were analyzed by SDS-polyacrylamide gel electrophoresis to look for induced protein bands. The poly(ADP-ribose) polymerase-deficient cell lines ADPRT54 and ADPRT351 clearly show increased protein levels at a molecular weight of 78,000. Fig. 3A also shows that the Mf, 78,000 protein band is increased in N3 cells grown in the absence of nicotinamide. In contrast, V79 cells and N3 cells grown in nicotinamide-containing regular medium do not exhibit any induction of these protein bands. Thus, cells deficient in poly(ADP-ribose) metabolism because of deficiency in enzyme or substrate clearly show increased levels of a protein at Mf, 78,000. However, upon growth in nicotinamide-containing regular medium, N3 cells restore their NAD level and resume poly(ADP-ribose) synthesis, and the elevated level of Mf, 78,000 protein decreases significantly.

The elevated protein band at Mf, 78,000 corresponds to one of the glucose-regulated proteins, GRP78. Usually induction of GRP78 is accompanied by induction of several other GRPs, of Mf, 94,000, 72,000, and 58,000, whose synthesis is coordinately regulated at the transcriptional level (26, 27); however, no increase in intensity was detected on the Coomassie blue-stained gel at these molecular weights. Since GRP78 is the most well characterized among all GRPs we subsequently focused our attention on this protein.

To further investigate the association of poly(ADP-ribose) synthesis deficiency with elevated levels of glucose-regulated proteins, we used a monoclonal antibody to GRP78 to examine the increased protein at Mf, 78,000. Cytosolic fractions of the different cell lines were analyzed by SDS-polyacrylamide gel electrophoresis and Western blot analysis. Fig. 3B shows the presence of GRP78 in all of the cell lines, as expected for a constitutively expressed protein. However, GRP78 is clearly overexpressed in the poly(ADP-ribose) polymerase-deficient cell lines ADPRT54 and ADPRT351 and also in the N3 cell line grown under conditions of nicotinamide deficiency.

Table 1 contains the results of densitometric scanning, showing that GRP78 levels increased by >3-fold in ADPRT54 and ADPRT351...
The other set of extracts was analyzed by SDS-polyacrylamide gel electrophoresis and subsequent Coomassie blue staining to look for induced protein bands as described in “Materials and Methods” (A). The other set of extracts was analyzed by SDS-polyacrylamide gel electrophoresis and subsequent Western blotting with anti-GRP78 monoclonal antibody as described in “Materials and Methods” (B).

Fig. 3. Cytosolic extracts prepared from V79, ADPRT54, ADPRT351, N3 cells grown in nicotinamide-deficient medium [N3 (−Nam)], and N3 cells grown in nicotinamide-containing regular medium [N3 (+Nam)] were divided into two sets. One set of extracts was analyzed by SDS-polyacrylamide gel electrophoresis and subsequent Coomassie blue staining to look for induced protein bands as described in “Materials and Methods” (A). The other set of extracts was analyzed by SDS-polyacrylamide gel electrophoresis and subsequent Western blotting with anti-GRP78 monoclonal antibody as described in “Materials and Methods” (B).

cells, compared to their parental V79 cells. N2, N3, and N4 cells grown under nicotinamide-deficient conditions also exhibited significant increases in GRP78 levels, compared to parental V79 cells. In contrast, N2, N3, and N4 cells grown in nicotinamide-containing regular medium showed GRP78 levels similar to those observed in the V79 cells. Thus, cells deficient in their ability to synthesize poly-(ADP-ribose) due to deficiency in enzyme or substrate clearly show increased levels of GRP78. However, upon restoration of NAD to normal levels, the overexpression of GRP78 disappears in N2, N3, and N4 cells.

Depletion of NAD Levels in V79 Cells and Its Effect on the Induction of GRP78 and the Cytotoxicity of VP-16. All of the studies described above were performed with cell lines selected for their ability to survive in the absence of the NAD-poly(ADP-ribose) stress response system. We considered the possibility that these cell lines may have had a stable alteration in regulation of GRP78 that allowed them to survive under this selective pressure. Thus, to eliminate the possibility of any artifact that may have resulted from long term growth of N2, N3, and N4 cells in NAD-deficient medium and to further investigate the relationship between NAD deficiency, induction of GRP78, and VP-16 resistance, we grew parental V79 cells in nicotinamide-deficient medium for time periods ranging from 0 to 3 days. At daily intervals, cells were trypsinized, resuspended in medium, and divided into three fractions. Samples were then analyzed for (a) NAD content, (b) GRP78 level, and (c) VP-16 sensitivity by clonogenic survival assays. Fig. 4A shows that, upon growth in nicotinamide-deficient medium for 1 day, NAD levels in V79 cells dropped by 71% and then continued to drop slowly on each consecutive day. Fig. 4B shows that GRP78 levels progressively increased upon growth of V79 cells in nicotinamide-deficient medium. On the basis of densitometric scanning, GRP78 levels were 1.13, 3.5, and 5.3 times higher in V79 cells grown in nicotinamide-deficient medium for 1, 2, and 3 days, respectively, compared to control cells grown in nicotinamide-containing regular medium. Fig. 4C shows that V79 cells developed progressive resistance to VP-16-induced cytotoxicity upon continuous growth in nicotinamide-deficient medium. Thus, these results further indicate an association between decreased NAD levels, elevated GRP78 levels, and increased resistance to VP-16-induced cytotoxicity.

Transcriptional Regulation of GRP78 in Poly(ADP-ribose) Polymerase-deficient Cell Lines. To evaluate whether overexpression of GRP78 was transcriptionally regulated, we measured the steady state level of GRP78 mRNA in the different cell lines by Northern blot analysis, using CHO-B as an internal standard. The results shown in Fig. 5 indicate that the steady state level of GRP78 mRNA is increased significantly in ADPRT54 and ADPRT351 cell lines. These levels are almost 5 times higher in mutant cells, compared to the parental V79 cells. Thus, induction of GRP78 in poly(ADP-ribose) polymerase-deficient cell lines appears to be controlled at the transcriptional level.

DISCUSSION

Our studies with poly(ADP-ribose) polymerase- and NAD-deficient cell lines demonstrate a clear association between the NAD and poly(ADP-ribose) polymerase stress response system, the GRP78 stress response system, and resistance to topoisomerase II-directed agents. We have shown that (a) cells that are unable to synthesize poly(ADP-ribose) based on enzyme or substrate deficiency are resistant to VP-16; (b) restoration of NAD levels in NAD-deficient cells results in reversal of resistance to VP-16; (c) GRP78 is overexpressed in cell lines deficient in poly(ADP-ribose) polymerase activity; (d) GRP78 is also overexpressed in cell lines deficient in NAD; (e) this overexpression of GRP78 is regulated at the transcriptional level; (f) restoration of normal NAD levels is followed by restoration of GRP78 to normal levels; (g) resistance to VP-16 is lost with concomitant loss of overexpression of GRP78; and (h) overexpression of GRP78 is associated with resistance to topo II-directed agents.

Interference with a variety of cellular processes can result in the induction of GRP78. Alteration of glucose metabolism by glucose deprivation or treatment with 2-deoxyglucose, depletion of calcium from intracellular calcium stores by calcium ionophore treatment, blocking of cellular glycosylation by agents such as tunicamycin, and exposure to glucosamine and prolonged anaerobiosis all result in the induction of GRP78 (26). Similarly, sulphydryl-reductive agents and low extracellular pH trigger the synthesis of GRP78 (26). Furthermore, interference with glycoprotein synthesis, as observed in somatic cell mutants defective in the glycosylation of cellular proteins and in cells infected with glycoprotein-containing paramyxoviruses, also induces GRP78 (26). In the present study, we have now demonstrated
that GRP78 overexpression can also be observed in cells deficient in poly(ADP-ribose) synthesis due to deficiency in either poly(ADP-ribose) polymerase or its substrate, NAD. However, our findings do not exclude the possibility of concomitant overexpression of the other GRPs under poly(ADP-ribose)-deficient conditions, because transcriptional regulation of the GRPs has been shown under other conditions to be tightly coupled (26, 27).

Previous studies have demonstrated a correlation between induction of GRP78 and VP-16 resistance. Our present studies extend these observations to demonstrate that the extent of VP-16 resistance is correlated with the levels of GRP78 protein, as well as with its induction. Although we have established an association between NAD and poly(ADP-ribose) synthesis deficiency, GRP78 overexpression, and resistance to VP-16, the mechanism of this association remains to be defined. Interactions between these systems may occur at the level of metabolic or protein interactions as well as at transcriptional and post-transcriptional regulatory levels. Because our studies show increased GRP78 mRNA in the poly(ADP-ribose) polymerase- and NAD-deficient cell lines, regulation at the transcriptional level must be given prime consideration. It has been shown that the rat GRP78 promoter contains multiple arrays of redundant regulatory elements that contribute to GRP78 induction by calcium ionophore A23187 (27, 28). Furthermore, using nuclear extracts from several mammalian cell lines, it has been demonstrated that multiple nuclear factors interact with various regions of the GRP78 promoter (29). Thus, it is possible that either poly(ADP-ribosylated) proteins or poly(ADP-ribose) itself may serve as a regulatory factor for synthesis of GRP78 and the absence of these regulatory factors could result in increased synthesis of GRP78. In this regard, it is interesting to note that poly(ADP-ribose) polymerase has previously been suggested to play a role in transcriptional regulation (2). It should also be noted that GRP78 exists primarily as an aggregated mono(ADP-ribosylated) protein in uninduced cells (30, 31). However, upon induction, GRP78 becomes monomeric and free of ADP-ribosylation (31). In addition, topoisomerase II has been shown to be poly(ADP-ribosylated) (32, 33) and, although the functional consequences of these post-translational modifications are unknown, it is possible that interference with these modifications could contribute to the alterations reported here. Furthermore, it should be pointed out that topoisomerase II and poly(ADP-ribose) polymerase exist in a nuclear environment (2, 3, 11, 13, 14), whereas GRP78 is a resident protein of endoplasmic reticulum (26). However, GRP78 in its induced form was found in the nucleus (26) and thus the possibility of its binding to other nuclear proteins and thereby inactivating them cannot be ruled out.

GRP78 has been shown to bind tightly to mutant proteins, to proteins with incorrect disulfide bonds, to incompletely assembled
multimeric proteins, and to glycoproteins with aberrant oligosaccharides (21, 34–39). As a result of this binding capacity, it has been proposed that GRP78 functions to retain mutated and malfolded proteins in the endoplasmic reticulum and prevent their secretion (21, 34–39). GRP78 synthesis can also be induced by treatment which results in misfolding of newly synthesized proteins, as well as by direct overexpression of mutant proteins which misfold (40, 41). These studies on the function of GRP78 have generally been focused on aberrant glycoproteins; however, it is possible that similar processes could occur with other proteins having abnormal tertiary structure due to interference with ADP-ribosylation and/or other post-translational modifications.

We also considered the possibility that GRP78 was induced in the ADPRT54 and ADPRT351 cells because the enzyme poly(ADP-ribose) polymerase is mutated in these cells, suggesting that induction of GRP78 in these cells may be associated with the presence of mutant or malfolded poly(ADP-ribose) polymerase. However, the fact that GRP78 is induced in poly(ADP-ribose) synthesis-deficient cell lines having low NAD levels and is reversed when the NAD level is restored suggests that the induction of GRP78 is associated with the functional decrease in poly(ADP-ribose) synthesis, rather than a direct structural abnormality of the protein. Since mono- and poly(ADP-ribosylation) is expected to be abnormal in the NAD-deficient cell lines, it is possible that loss of this post-translational modification results in structural abnormalities that induce the GRP78 system.

Clearly, the mechanism of this association will require further study.

Several pathways have now been shown to account for resistance to topoisomerase II-directed agents, and all of these may have clinical importance in modulating responsiveness to cancer chemotherapy. These include direct mutation in the topoisomerase II gene, which can alter binding characteristics of the enzyme, leading to drug resistance (12, 42, 43). A second cause of resistance to topo II-directed agents is the multiple drug resistance phenotype mediated by overexpression of the P-glycoprotein, which essentially acts to “pump” certain topo II-directed agents out of the cell (44, 45). A third mechanism to confer resistance to topo II-directed agents is associated with induction of stress-responsive proteins such as GRP78 by a variety of agents, as discussed above (19, 20).

Although the resistance to VP-16 and other topo II-directed agents that we have reported in this study is associated with induction of the glucose-regulatory stress response proteins, our results suggest that a fourth category of variable nutrition-induced resistance should be considered for VP-16 and other topo II-directed agents. Thus, nicotinamide and niacin, which serves as the major dietary precursor for NAD, are essential nutritional elements. Severe nutritional deficiency of niacin and nicotinamide has been clearly documented as the cause of pellagra. However, recent studies suggest that subclinical deficiencies of niacin associated with low NAD levels may be common (46). Moreover, nicotinamide wasting has previously been noted as part of the catabolic processes associated with progressive tumor growth (47, 48). Thus, certain tumors which are ordinarily responsive to topo II-active agents may become resistant due to variable niacin or nicotinamide nutritional status and decreased NAD levels in the tumor. In our studies with V79 cells, exposure to conditions of nicotinamide deficiency for only 24 h produced a significant decrease in NAD levels and increased resistance to VP-16. Thus, poly(ADP-ribose) polymerase and NAD levels as well as niacin nutritional status may be important factors to study in determining the etiology of variable sensitivity to VP-16 and other topo II-directed agents.

REFERENCES


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