NAD(P)H:Quinone Oxidoreductase Gene Expression in Human Colon Carcinoma Cells: Characterization of a Mutation Which Modulates DT-Diaphorase Activity and Mitomycin Sensitivity

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ABSTRACT

NAD(P)H:quinone oxidoreductase (DT-diaphorase; DTD) is an obligate two-electron reductase which may play a role in the bioactivation of antitumor quinones such as mitomycin C (MMC). We studied 10 colon carcinoma cell lines showing different levels of DTD activity (range, 0–3447 nmol/min/mg protein), as measured by the reduction of dichlorophenolindophenol. Expression of the NAD(P)H:quinone reductase gene (NQO1), which codes for the DTD enzyme, as measured by a polymerase chain reaction amplification technique was then correlated with enzymatic activity in all cell lines. HT-29 cells, which have intermediate DTD activity (769 ± 144 nmol/min/mg protein, mean ± SD) and are sensitive to MMC, showed high NQO1 expression relative to β-actin (taken as 100% here for comparative purposes). BE cells which have no detectable DTD activity and are resistant to MMC showed moderate NQO1 expression (91% of HT-29). RNA single-strand conformational polymorphism analysis and subsequent sequencing of BE complementary DNA revealed a C to T mutation in the NQO1 complementary DNA. This confers a proline to serine substitution in the amino acid sequence of the protein. Additionally, Idl-11 h cells showed both moderate DTD activity (390 ± 41 nmol/min/mg protein) and NQO1 expression (41% of HT-29), while resistant subclones of these cells, exposed to MMC during 11 and 44 weeks, showed low gene expression (5% and 9% of HT-29 respectively) and enzymatic activity (11 ± 6 and 36 ± 16 nmol/min/mg protein). These results support the ideas that reductive activation of MMC by DTD may be important in the cytotoxicity of MMC and that polymerase chain reaction may be a useful technique for quantitating the relative expression of genes in human tumors.

INTRODUCTION

Antitumor quinones, such as MMC and AZQ, require enzymatic reduction prior to eliciting their antitumor activity (1, 2). These quinones, however, can undergo either one-electron reduction to the semiquinone or two-electron reduction to the hydroquinone, and it is not clear which metabolic pathway is responsible for the generation of the ultimate cytotoxic species (3–6). Reduction of either the semiquinone or the hydroquinone can lead to the production of reactive oxygen species and DNA fragmentation (4–6). Two-electron reduction initially forms the hydroquinone species, and in the case of MMC and AZQ, this species is more potent alkylating agent than the parent quinone. The antitumor activity of any given quinone is, therefore, dependent upon the reductive capacity of the tumor and upon the cytotoxic potential of the reduced products.

A major two-electron reductase in biological systems is NADPH-quinone oxidoreductase or DT-diaphorase under aerobic conditions. BE and HT-29 cells are both colon carcinomas, and one may speculate that the clinical utility of either MMC or AZQ against colon carcinomas may be a reflection of DTD activity within the tumor when compared with the surrounding colonic mucosa.

In this study, we measured DTD both by an enzymatic assay and by a PCR assay designed to quantitate gene expression. The results show that in 10 human colon carcinoma cell lines the level of DTD varies considerably. Surprisingly, BE cells were found to have high levels of gene expression but no enzymatic activity. Sequence analysis of the PCR products showed that the BE cells have a mutation in the NADPH-quinone oxidoreductase (NQO1) gene. A striking feature observed in the other 9 cell lines was the excellent agreement between the PCR-based assay and the enzymatic activity. These data illustrate the usefulness of the PCR as a technique to measure relative gene expression, and in the case of DTD the level of gene expression reflects enzymatic activity.

MATERIALS AND METHODS

Chemicals and Enzymes. M-MLV reverse transcriptase, and T7 RNA polymerase were obtained from BRL, Gaithersburg, MD; [α-35S]dATP (1207 Ci/mmol) and [α-32P]GTP (1019 Ci/mmol) were obtained from Dupont NEN, Boston, MA; [α-32P]CTP (3000 Ci/mmol) was obtained from Amer sham, Arlington Heights, IL; recombinant Taq DNA polymerase (AmpliTaq) was obtained from Perkin Elmer Cetus, Norwalk, CT; RNA guard RNase inhibitor, ribonucleoside-5'-triphosphates, random hexamers, deoxynucleoside-5'-triphosphates, and DNA polymerase I (Klenow fragment) were obtained from Pharmacia. Recombinant T7 DNA polymerase (Sequenase version 2.0) sequencing kit was obtained from USB, Cleveland, OH. Dicumarol and DCPIP were obtained from Sigma. Except where noted, all other chemicals and buffers used were of molecular biology grade.
Cell Lines. HT-29 and BE colon carcinoma cells have been cultured in this laboratory for a number of years. HCT-116, HCT-116/11, and HCT-116/44 colon carcinoma cells were kindly provided by Dr. Michael Brattn, Baylor School of Medicine, Houston, TX. HCT8(S) and HCT8(R) were obtained from Dr. Kevin Scanlon, City of Hope Medical Center, Duarte, CA. All other cell lines were obtained from American Type Culture Collection, Rockville, MD. HT-29, BE, HCT-116, HCT-116/11, HCT-116/44, HCT8(S), and HCT8(R) were maintained by growing cells at 37°C as monolayers in Eagle’s minimum essential media supplemented with 10% calf bovine serum, gentamicin (0.05 mg/ml); glutamine (0.03 mg/ml), 0.1% nonessential amino acids, 0.1 mM sodium pyruvate, and 0.02 M (2-hydroxyethyl)-1-piperazineethanesulfonic acid. LS174T cells were maintained in Eagle’s minimum essential media supplemented with fetal bovine serum. Lysates were prepared from 300 million cells in 320DM and COLO 320HSR cells were maintained by growing cells in RPMI 1640 media supplemented with 10% calf bovine serum, glutamine (0.03 mg/ml), and kanamycin (0.1 mg/ml).

Enzymatic Activity of DTD. Cells were grown to 80% confluence before being washed with Hank’s balanced salt solution and scraped into ice cold buffer [25 mM Tris-HCl (pH 7.4) and 250 mM sucrose]. A cell sonicate was then made for each colon cancer cell line by sonicating the cell suspension for 30 s on ice. DTD activity of the cell sonicates was then assayed according to Ernster (9) as modified by Benson et al. (10). Reactions (0.5 ml) were performed at 25°C in the presence and absence of 0.02 mM dexamethasone in a buffer containing 25 mM Tris-HCl (pH 7.4), 0.7 mg/ml bovine serum albumin, 0.2 mM NADH, and 0.04 mM DCPIP. DTD activity was measured as the dexamethasone-sensitive reduction of DCPIP (21,000 M⁻¹ cm⁻¹) measured by the decrease in absorbance at 600 nm during 30 s in a Shimadzu UV160U spectrophotometer. Protein content in the cell sonicates was assayed by the method of Bradford (11).

Isolation of Total RNA from Human Tumor Cells. For PCR quantitation, RNA was isolated by the method of Chomzynski and Sacchi (12). For sequencing, RNA was isolated essentially as described by Huang and High (13). The cells were grown to 80% confluence, the culture medium was removed, and the cells were washed twice with phosphate-buffered saline and lysed directly on the plates in 6 ml of solution 1 (2% sodium dodecyl sulfate, 200 mM Tris-HCl, 1 mM phosphate-buffered saline and lysed directly on the plates in 6 ml of solution 1 (2% sodium dodecyl sulfate, 200 mM Tris-HCl, 1 mM EDTA). The resulting lysate was drawn slowly through a 20-gauge needle four times and divided equally into six 1.5-ml centrifuge tubes. To each tube, 300 μl of ice cold solution 2 (42.9 potassium acetate, 11.2 ml acetic acid in water to 100 ml) was added before precipitating the RNA fragments on ice for 2 min. Following precipitation, the tubes were centrifuged at 14,000 rpm for 7 min at 25°C. The resulting pellets were combined, washed with 70% ethanol, recentrifuged briefly, dried under vacuum for 20 min, and resuspended in 130 μl of RNase-free water.

Reverse Transcription of RNA. The isolated RNA was immediately reverse transcribed to cDNA as recommended by BRL. Briefly, 120 μl of the RNA solution was used in a reaction containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 10 mM dithiothreitol, 3 mM MgCl₂, 0.5 mM dithiothreitol, 3.5 units/ml of RNase inhibitor, 0.25 units/ml of random hexamers, and 10 units/ml of MMLV-RT. This solution was incubated at 37°C for 1 h and stopped by heating to 95°C for 5 min. The resulting cDNA was then stored at -20°C for further use. For the quantitation of NQO1 expression, minor modifications in the reverse transcription procedure were used as described by Horikoshi et al. (14).

Quantitation of NQO1 Expression by PCR. NQO1 expression was measured by the PCR-based method developed by Horikoshi et al. (14). Varying amounts of cDNA from each cell line were amplified in 25 μl of reactions containing 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 0.2 mM deoxynucleotide triphosphates, 1.875 mM MgCl₂, 0.625 units of AmpliTaq polymerase, and 12.5 pmol of each primer (synthesized using an Applied Biosystems model 391 PCR-MATE DNA synthesizer by the phosphoramidite method) (see Table 1 for primer sequences).

### Table 1 Primers used in PCR and sequencing reactions (14)

<table>
<thead>
<tr>
<th>Primer</th>
<th>Description</th>
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<tbody>
<tr>
<td>ASN776: TTT CTC CTC ATC CTG TAC C</td>
<td>(776-795 of NQO1)</td>
</tr>
<tr>
<td>ASN673: TAT TCT CCA CGC GTT TCT TCC</td>
<td>(673-694 of NQO1)</td>
</tr>
<tr>
<td>ASN918: GAA GCC TGG AAA GAT ACC C</td>
<td>(918-937 of NQO1)</td>
</tr>
<tr>
<td>SN241: ACT TTC ACT ATC CTG CCG</td>
<td>(241-259 of NQO1)</td>
</tr>
<tr>
<td>SN667: AGG ATG GAA ACG CCT GG</td>
<td>(667-687 of NQO1)</td>
</tr>
<tr>
<td>SN439: CTG CCA TGT ATG ACA AAG GAC C</td>
<td>(439-461 of NQO1)</td>
</tr>
<tr>
<td>SN657: GAG ATG GAA GCA ACG TCC G</td>
<td>(657-676 of NQO1)</td>
</tr>
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Thermal cycling followed the pattern of 1-min denaturation at 93.5°C, 1-min annealing at 55°C, and 1-min extension at 72°C in an Epicomp Twin Block (San Diego, CA) thermal cycler. Amplifications were carried out for 30 cycles, with the exception of a longer annealing step (8 min) on the last cycle. A T7 RNA polymerase promoter sequence (TAA TAC GAC TCA TGT TA) was included on the 5’ end of the antisense primer (see Table 1). Following amplification of the cDNA, 3 μl of the resulting fragments was then transcribed to RNA in a 25-μl reaction containing 40 mM Tris-HCl (pH 7.5), 12 mM MgCl₂, 10 mM dithiothreitol, 2 mM spermidine, 0.39 units of RNA guard, 1 mM ribonucleotides, and 0.25 μl of [α-³²P]CTP, by 0.68 μl T7 RNA polymerase (69 units/μl). This mixture was incubated for 1 h at 37°C and stopped by the addition of 0.75 μl of 0.5 mM EDTA. The radiolabeled RNA fragments were then isolated on a 5% polyacrylamide/8 M urea gel and quantitated by liquid scintillation counting of the excised fragments. For each cell line, an endogenous standard (either β-actin or βM) was also amplified from the cDNA. The amount of PCR products used as a reference for the starting amount of DNA is described by the equation: N = N₀(1 + eff)ᵗ, where N₀ is the number of copies of the starting DNA, N is the amount of DNA after n cycles of amplification, and eff is the efficiency of the amplification (14). For each cell line, a range of cDNA concentrations over which the amplifications resulted in a linear increase in incorporated radioactivity was established. To normalize the NQO1 expression to that of the endogenous standards, the ratio between the amount of the radiolabeled PCR product within the linear amplification range of the target gene and the endogenous standard was calculated as follows:

\[
\frac{\text{Ratio of PCR products}}{\text{Volume of target gene}} = \frac{\text{cpm target gene}}{\text{Volume of internal control gene}} \times \frac{\text{Volume of internal control gene}}{\text{Volume of target gene}}
\]

Because HT-29 colon carcinoma cells showed both intermediate enzymatic activity and gene expression, we chose these cells as standards against which the remaining carcinoma cells were compared.

SSCP Analysis of DTD Gene Fragments. RNA SSCP was carried out essentially as described by Danenberg et al. (15). RNA fragments were generated by transcribing the same fragments used for the quantitation of gene expression in the presence of [α-³²P]GTP using T7 RNA polymerase obtained from BRL according to the manufacturer’s recommendations. Three μl of final RNA solution was then run on a 7% polyacrylamide gel (dimensions, 160 × 160 × 1.5 mm) at 60 V for 12 h at 4°C. The gel was then dried and exposed to Kodak XAR
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film overnight at room temperature.

Sequencing of PCR Products. Single-strand DNA fragments for sequencing were created and isolated by asymmetric amplification of aliquots of the cDNA as described previously (16). Sequencing of the amplified cDNA from BE and HT-29 cells was performed using a modification of the Sanger method (17). Briefly, single-strand DNA was resuspended in 6 μl of water, 2 μl of dimethyl sulfoxide, 2 μl of Sequenase buffer, and 1 pmol of a sequencing primer (see Table 1), heated to 65°C for 2 min, and then cooled to room temperature for 45 min. To this mixture, 1 μl of 0.1 M dithiothreitol, 2 μl of 5-fold diluted labeling mix, 0.5 μl of [α-35S]dATP, 1 μl of Mn buffer (0.15 M sodium isocitrate and 0.1 M MnCl₂), and 2 μl of 8-fold diluted Sequenase version 2.0 T7 DNA polymerase were added. This mixture was incubated for 2 min at room temperature before 3.5-μl aliquots were added to 2.5 μl of each dideoxy termination mixture. These tubes were then incubated at 48°C for 8 min, at which point 2 units of Klenow polymerase was added. The termination reactions were then incubated for an additional 3 min, stopped by the addition of 4 μl of stop mixture, and denatured at 75°C for 3 min prior to electrophoresis on a 4 M urea/8% polyacrylamide gel.

RESULTS

Gene Expression and Enzymatic Activity. Fig. 1 shows an autoradiogram of a 248-nucleotide NQO1 fragment amplified from a broad concentration range of cDNA isolated from HT-29 cells. The intensity of this band increases as the starting volume of cDNA is increased in the PCR amplification assay. These bands were excised and the radioactivity measured to establish the relationship shown in Fig. 2. As the graph indicates, the linear range decreases between 0 and 5 nl of cDNA, and the inset shows an expansion of this linear range which has a correlation coefficient of 0.96. Similarly, Fig. 3 shows an autoradiogram of an amplified actin fragment, and Fig. 4 shows the subsequent linear portion of both β-actin and β₂M amplifications using HT-29 cDNA which have r values of 0.99 and 0.98, respectively. In the same manner, the linear range for NQO1 and the two endogenous standards was then established for each of the 10 colon carcinoma cell lines tested.

As described in “Materials and Methods,” these linear ranges were then used to normalize the NQO1 counts relative to the counts of the endogenous standard (14). The results of this

Fig. 1. Autoradiogram of NQO1 RNA fragment amplified and transcribed from varying amounts of HT-29 colon carcinoma cDNA.

Fig. 2. Relationship between volume of HT-29 cDNA amplified over 30 cycles and radioactivity of the resulting RNA fragments. Inset, expansion of the linear range and correlation coefficient for this portion of the graph.

Fig. 3. Autoradiogram of β-actin RNA fragment amplified and transcribed from varying amounts of HT-29 colon carcinoma cDNA.

Fig. 4. Linear range of cDNA amplification and transcription.
calculation and the enzymatic activity for each cell line is shown in Table 2. The range of enzymatic activity among the 10 different cell lines (0-3447 nmol/min/mg protein) is large, as is the relative gene expression. The HCT8 (R) and HCT8(S) cells show enzymatic activity of 3447 and 2759 nmol/min/mg protein, respectively. This is 4.5- and 3.6-fold higher than the enzymatic activity observed with HT-29 cells and is in excellent agreement with the 4.5- and 5.7-fold higher levels of NQO1 expression relative to β-actin (Table 2). HT-29, HCT-116, and LS174T cells all show moderate DTD activity and gene expression. COLO320HSR, COLO 320DM, HCT-116/11, and HCT-116/44 cells show enzymatic activity <9% of that found in HT-29 cells. Finally, the BE cells have no detectable enzymatic activity, yet a moderately high level of gene expression relative to the HT-29 cells was observed. When the BE cells are excluded, however, a correlation coefficient of 0.96 is observed between the levels of enzymatic activity and the relative levels of gene expression (Table 2).

RNA SSCP Analysis of NQO1 Fragments. To explore the reason for the disparity in NQO1 expression and enzymatic activity in the BE cells, we used RNA SSCP analysis to search for mutations and/or deletions in the NQO1 cDNA isolated from these cells (Fig. 5). The two major bands in each lane are presumed to arise from two distinct conformations of the 248-base NQO1 RNA fragment which exist in equilibrium but move through this nondenaturing gel with different mobilities. In the lanes containing the HT-29, HCT-116, HCT-116/11, and HCT-116/44 RNA fragments, the mobility of the two major bands was identical. RNA synthesized from BE cDNA showed a subtle but reproducible change in the mobility of the two conformations of the 248-nucleotide NQO1 RNA fragment. This was distinct from that observed in the other cell lines in that the lower band migrated more slowly (Fig. 5), suggesting the presence of a mutation in this fragment.

Sequencing of BE and HT-29 NQO1 Fragments. Single-strand DNA for sequencing was generated from HT-29 and BE cells as described in "Materials and Methods." This DNA then served as a template for enzymatic sequencing of the 5′-untranslated region (encompassing the 38 nucleotides immediately 5′ of the initiation codon) as well as the coding region of the NQO1 cDNA. Fig. 6 shows a C to T point mutation present in the BE NQO1 cDNA which corresponds to a proline 187 to serine 187 change in the resulting DTD enzyme in these cells. This single-base change in the BE cDNA occurs in the fragment which displayed different mobility in SSCP analysis and is presumably responsible for the observed changes. The mutation was evident when sequencing was conducted using either a sense or antisense NQO1 template or by sequencing from RNA fragments using reverse transcriptase (18) (data not shown). Identical results were obtained with multiple amplifications and sequencing, suggesting that the mutation is not the result of misincorporation by the AmpliTaq polymerase but represents the true BE sequence. The mutation occurs at position 609 of the human liver NQO1 cDNA described earlier and, with the exception of one degenerate change (a G to C substitution at threonine 98), the sequence in both the HT-29 and BE cells otherwise matches the published sequence of the human liver NQO1 (19).

### DISCUSSION

Using 10 human colon carcinoma cell lines, we have shown that the relative gene expression of NQO1 can be quantitated by a PCR-based method. Similar quantitation methods have been used with various strategies to control for differences inherent in amplification efficiency, RNA recovery and subsequent degradation, starting cell number, and the extent of reverse transcription (14, 20–24). In many of these studies, β2M

| Table 2 Comparison of NQO1 expression versus enzymatic activity in 10 human colon carcinoma cell lines |
|------------------|------------------|------------------|
| Cell line       | Gene expression relative to HT-29 | DTD/β-actin | DTD activity relative to HT-29 |
|                 | DTD/β-M          | DTD/β-M         |
| HT-29           | 1.00             | 1.00            | 1.00 (769) |
| BE              | 0.91             | 0.72            | 0.00 (0)   |
| HCT-116         | 0.41             | 0.60            | 0.51 (390) |
| HCT116/11       | 0.05             | 0.02            | 0.02 (12)  |
| HCT116/44       | 0.09             | 0.05            | 0.05 (36)  |
| LS174T          | 0.15             | 0.46            | 0.31 (235) |
| COLO 320 HSR    | 0.013            | 0.01            | 0.01 (8)   |
| COLO 320 DM     | 0.015            | 0.01            | 0.005 (4)  |
| HCT 8 (S)       | 5.7              | 75.2            | 3.59 (2759)|
| HCT 8 (R)       | 4.5              | 125             | 4.48 (3447)|

* A good correlation (r = 0.96) is observed between levels of gene expression relative to β-actin and relative enzymatic activity.

* Values in parentheses, actual enzymatic activity expressed as nmol/min/mg protein.
compared. In the HCT8(R) and HCT8(S) cells, for instance, was used as an endogeneous standard against which the relative /3-actin may be a more reliable standard in studies utilizing suggested an inordinately high level of NQO1 expression. This when NQO1 expression and DTD enzymatic activity were as an endogenous standard produced the most consistent data endogeneous standard (25). In our studies, ß-actin expression a C to T point mutation at position 609 of the NQO1 cDNA of the BE cell. expression in these cells. Similar levels of enzymatic activity exposure to the drug during 11 or 44 weeks, respectively (26). good correlation between gene expression and enzymatic activ were found in these cells after at least 30 cell doublings (data not shown). This data is consistent with the previous work by Willson et al. (26) which suggested that MMC resistance is stable for at least 1 year in these cells. When we screened fragments of the NQO1 cDNA via RNA SSCP, the 248-base fragment shown in Fig. 5 was found to differ in the BE cells. RNA can assume many different conformations which exist in equilibrium with each other. The 248-base RNA fragment analyzed here appears to assume two major conformations. These two conformations impart a different mobility in the gel matrix to the RNA fragment giving rise to the two major bands readily apparent on the autoradiogram. Mutations, deletions, or insertions in this strand of RNA which result in new conformations in the fragment can then be detected by the altered mobility of the new conformations in the gel. This is the case with the RNA fragment produced from cDNA from BE cells. The subtle differences in mobility of the two major conformations of this RNA fragment are indicative of differences in the NQO1 cDNA in BE cells when compared with the same fragment from the HT-29 cells. The usefulness of RNA SSCP as an indicator of single-point mutations is thus evident from our results and provides a clue as to the undetectable enzymatic activity in the BE cells.

Sequencing of the coding region from both BE and HT-29 cDNA identified a C to T point mutation in the NQO1 gene. This results in the conversion of proline 187 to serine 187 in the DTD enzyme in the BE cells. This amino acid change is presumably responsible for the lack of enzymatic activity observed in these cells. This point mutation, however, did not alter the ability of BE cells to synthesize NQO1 mRNA. Additionally, we have found that the sequence in the 5'-untranslated region of the NQO1 (at least for the 38 nucleotides immediately 5' of the initiation codon) is identical in BE and HT-29 cDNAs and is in agreement with that previously reported by Jaiswal et al. (19). This suggests that ribosome binding, and thus translation, of the NQO1 mRNA in the BE cells is likely to be no different than that in HT-29 cells (27). At this time, however, we have been unable to determine whether the DTD protein is present or absent in the BE cells. This is the first mutation identified in the coding region of the NQO1 gene and may provide information about the amino acid sequence required for enzymatic function. The loss of a proline residue in the enzyme is itself significant because this amino acid confers major secondary structural changes in a protein due to the rigidity of the proline ring (28). This may result in the disruption of α helix or β-sheet structures in the region of the protein affected. Whether this is the reason for the loss of enzymatic activity in the BE cells remains to be elucidated.

Recently, an area associated with the pyridine nucleotide-binding site of the rat hepatic DTD dimer was determined by the use of photoaffinity analogue of 4-hydroxycoumarin (29). This region is near the proline 187 to serine 187 substitution which we believe is responsible for the loss of enzymatic activity in BE cells. It is possible that the conformational changes resulting from the substitution of this serine residue could adversely affect the pyridine-binding site in the enzyme. Additionally, this serine substitution occurs just six residues from the only cysteine amino acid in the enzyme. This may be significant in that conformational changes around the lone cysteine may alter the ability of this residue to function in an active site of the enzyme or participate in the dimerization of the DTD monomers. This close spatial relationship between

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**Fig. 6. Antisense sequencing of HT-29 and BE colon carcinoma cDNA. Arrows, a C to T point mutation at position 609 of the NQO1 cDNA of the BE cell.**
the cysteine and altered proline residues in the BE cell DTD and the apparent absence of any enzymatic activity in these cells then suggests that this region of the protein may be crucial to the proper enzymatic functioning of DTD.

The associated insensitivity of the BE cells of MMC (2) further supports the involvement of DTD in the activation of the drug. Also, the absence of NQO1 expression in HCT-116 cells which are resistant to MMC (26) provides additional evidence that DTD is necessary for cytotoxicity of MMC. This observation suggests the isolation of two mutant cell lines resistant to MMC due to decreased NQO1 expression. An explanation for the lower NQO1 expression or down regulation of this gene in these colon carcinoma cells is currently being pursued in our laboratory.

The good agreement between the enzymatic activity and the relative level of gene expression in the tumor cell lines studied suggests that further use of the PCR technique used in this study may be warranted. It may be possible to successfully quantitate the relative gene expression of many proteins in patient tumor samples, making it possible to correlate the observed clinical response to MMC with NQO1 expression in these cancer cells. Indeed, this technique has been successfully utilized to evaluate the relative gene expression of the thymidylate synthase and dihydrofolate reductase genes (14). The potential application of this PCR technique in terms of predicting MMC sensitivity is supported by the observations that the DTD activity is higher in colon carcinomas than in the surrounding colonic mucosa (30).

REFERENCES

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