Expression of a mutant DNA topoisomerase II in CCRF-CEM human leukemic cells selected for resistance to teniposide

(atypical multidrug resistance/ATP-binding fold/point mutation)

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Communicated by Robert T. Schimke, May 17, 1991 (received for review December 18, 1990)

ABSTRACT Nuclear extracts from teniposide (VM-26)-resistant sublines of the human leukemic cell line CCRF-CEM have decreased levels of DNA topoisomerase II catalytic activity and decreased capacity to form drug-stabilized covalent protein–DNA complexes. The ATP concentration required for equivalent activity in a DNA-unknotting assay is 2- to 8-fold higher in nuclear extracts from drug-resistant cell lines as compared with the parental line. When adenosine 5'-[β , γ -imido]triphosphate is substituted for ATP in complex-formation assays, no significant change is seen with drug-sensitive cells, but a 50–65% reduction is seen with VM-26-resistant cells. Collectively, these results indicate that an alteration in ATP binding may be involved in the resistance phenotype. Therefore, we identified regions of the topoisomerase II sequence that conform to previously identified nucleotide-binding sites. Starting with cDNA as the template we determined the sequence of the topoisomerase II mRNA surrounding these sites by sequencing DNA fragments produced by the polymerase chain reaction. In the region corresponding to the consensus B ATP-binding sequence described by Walker *et al.* [Walker, J. E., Saraste, M., Runswick, M. J. & Gay, N. J. (1982) *EMBO J.* 1, 945–951], the cDNA from the two VM-26-resistant sublines contained an altered sequence having a G $\rightarrow$ A base change. This base substitution results in the replacement of the conserved arginine at position 449 with a glutamine. Hybridization with allele-specific oligonucleotides confirmed the presence of both the normal and the altered sequence in the resistant cell lines, whereas only the normal sequence was found in the sensitive CEM cells. A chemical mismatch cleavage procedure for the detection of mispaired bases in DNA duplexes identified no other alterations in the 5' third of the mRNA coding sequence, which contains the complete ATP-binding domain of topoisomerase II. The presence of mRNA encoding topoisomerase II with Gln⁴⁴⁹ correlates both with the presence of a topoisomerase II protein whose interaction with ATP is altered and with increased resistance to the cytotoxicity of VM-26.

DNA topoisomerase II is an essential nuclear enzyme that catalyzes the interconversion of topological forms of double-stranded DNA (1–3). This activity is required for DNA replication, recombination, and chromosome segregation (1, 4). The cDNA sequence of a topoisomerase II from HeLa cells (5) corresponded to a 174-kDa protein (170-kDa form). A second distinct form of topoisomerase II having an apparent molecular mass of 180 kDa has been identified (6). The 170-kDa form is more sensitive to the topoisomerase II inhibitors teniposide (VM-26) and merbarone than the 180-kDa form and the two forms differ in their cleavage site, thermal stability, and inhibition by A+T-rich oligonucleotides (7). Chung *et al.* (8) have isolated partial cDNAs specific

for two topoisomerase II proteins. The sequence of one cDNA, topoisomerase II α , is identical to the sequence previously published for topoisomerase II (5). The other cDNA codes for the similar but distinct topoisomerase II β protein. Our report analyzes only the topoisomerase II α sequence (170-kDa form). The nucleotide and amino acid numberings used are as in ref. 5.

Several classes of antitumor drugs, including the anthracyclines, epipodophyllotoxins, and aminoacridines, inhibit the catalytic activity of topoisomerase II (9–14), and both rodent and human cell lines have been selected for resistance to these drugs (15–21). In most cases cells that have been selected for resistance to a single topoisomerase II-inhibiting drug are cross-resistant to drugs of the other classes. This type of multidrug resistance, termed at-MDR, has been associated with an altered topoisomerase II activity (22–25) or a decrease in the amount of the enzyme (26). Previous studies of the human leukemic cell line CCRF-CEM and two VM-26-resistant sublines showed that topoisomerase II in nuclear extracts from resistant cells required a higher concentration of ATP than an equal amount of topoisomerase II from sensitive cells to achieve equivalent P4 DNA unknotting (20, 25). Also, only with extracts from the sensitive cells could adenosine 5'-[β , γ -imido]triphosphate substitute for ATP to increase covalent topoisomerase II–DNA complexes in the presence of VM-26 or 4'-(9-acridinyl)aminomethanesulfon-*m*-anisidine (*m*-AMSA). To characterize this altered ATP interaction at the DNA level, consensus sites for nucleotide interaction were identified in the topoisomerase II sequence, and the sequences flanking these sites in the VM-26-resistant cells were determined. A single base change was identified by comparison with the wild-type topoisomerase II sequence.

MATERIALS AND METHODS

Cell Culture and Nucleic Acid Isolation. CEM and VM-26-resistant cells were cultured and selected as described (20, 27). DNA (28), total cellular RNA (29), and poly(A)⁺ mRNA (30, 31) were prepared as described. cDNA was synthesized from 1–5 μ g of poly(A)⁺ mRNA with 3' specific or random primers by using the Lambda Librarian kit (Invitrogen, San Diego).

Synthesis and Isolation of Polymerase Chain Reaction (PCR) Products. One-tenth of the first-strand cDNA preparation was added to PCR mixtures containing 100 pmol of each specific primer and 200 μ M dNTPs in 50 μ l of reaction buffer (50 mM KCl/10 mM Tris Cl, pH 8.3/1.5 mM MgCl₂/0.01% gelatin). The mixture was heated to 95°C for 3 min and cooled

Abbreviations: ASO, allele-specific oligonucleotide; *m*-AMSA, 4'-(9-acridinyl)aminomethanesulfon-*m*-anisidine.

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to room temperature before addition of 2.5 units of AmpliTaq DNA polymerase (Perkin-Elmer/Cetus). Either of two different temperature programs was used: (i) 30 cycles of 37°C for 2 min, 72°C for 5 min, and 94°C for 1 min, followed by 1 cycle of 37°C for 2 min and 72°C for 10 min, or (ii) 35 cycles of 50°C for 30 sec, 72°C for 1 min, and 93°C for 30 sec, followed by 1 cycle of 50°C for 2 min and 72°C for 10 min. After agarose gel electrophoresis, PCR products were visualized by ethidium bromide staining, and the proper-sized fragments were sliced out. The position and sequence of the PCR primers are shown in Fig. 1.

Subcloning and Sequencing of PCR Products. In some experiments, gel-purified PCR products were sequenced directly by a modification (38, 39) of the dideoxy chain-termination procedure (40) with a Sequenase kit (United States Biochemical). When subcloning before sequencing was required, gel-purified PCR products were digested with *Pst* I or *Pst* I/*Hind*III as appropriate, and the proper-sized DNA bands were sliced from agarose gels. Ligations with digested M13mp18 phage DNA were performed with the agarose present. *Escherichia coli* JM101 cells were transformed with 3 μ l of each ligation mixture by the CaCl_2 method (41) and plated with 5-bromo-4-chloro-3-indolyl β -D-galactopyranoside for determination of recombinant plaques. Plaques were screened for topoisomerase II inserts by standard nitrocellulose plaque-lift methods (31) with hybridization to the ^{32}P -labeled 3-kilobase-pair (kbp) *Eco*RI fragment of the topoisomerase II cDNA hTOP2 (generously supplied by L. F. Liu, Johns Hopkins University School of Medicine). In forced-cloning experiments, 12–50 positive clones from a cell line were combined for production of a consensus sequence. DNA sequencing was performed by the dideoxy chain-termination method (40) with a Sequenase kit.

Allele-Specific Oligonucleotide (ASO) Hybridization. Oligonucleotides of 21 bp were synthesized to correspond to the sequence surrounding bp 1346 in either the wild-type (TOPII-ASO1, AGTGGAGTTTCGGCCCCCTGC) or the resistant (TOPII-ASO2, AGTGGAGTTTGGCCCCCTGC) cell DNA. The oligonucleotides were end-labeled with [γ - ^{32}P]dATP by polynucleotide kinase. Hybridizations of ASOs to PCR products were performed at 2°C below the calculated melting temperature in 5 $\times$ SSPE/0.5% SDS/5 $\times$ Denhardt's reagent (42) (1 $\times$ SSPE is 20 mM sodium phosphate, pH 7.4/0.18 M NaCl/1 mM Na_2EDTA , pH 8.0). The filters (Duralon UV; Stratagene) were washed at the melting temperature in 2 $\times$ SSPE/0.1% SDS for 10 min. After autoradiography, the first oligonucleotide was "stripped off" the

FIG. 1. Position of the oligonucleotide primers used in PCRs to generate specific DNA fragments. Underscored bases have been changed from the normal sequence to create the indicated restriction sites. Consensus sequences are shown as black boxes at their approximate positions in the topoisomerase protein: 1, consensus A sequence of ATP-binding fold (32); 2, nuclear targeting site (33); 3, consensus B sequence of ATP-binding fold (32); 4, topoisomerase II signature sequence; 5, dinucleotide-binding $\beta\alpha$ unit (34); 6, reactive tyrosine in transient covalent bond to DNA (35, 36); 7, leucine zipper (37).

filter as suggested by the manufacturer and the filter was hybridized to the second oligonucleotide under similar conditions. The filter was washed and autoradiographed as before.

Chemical Mismatch Cleavage. A modification of the procedure of Grompe *et al.* (43) was used to analyze the topoisomerase II sequence for base-pair changes by the chemical mismatch cleavage procedure of Cotton *et al.* (44). The primers (OTOPII-5.2 and OTOPII-3.4) used for synthesis of the labeled PCR fragments yield a 1529-bp product (positions 287–1815; Fig. 1).

RESULTS

Our previous studies indicated that an alteration existed in the interaction of ATP and topoisomerase II in nuclear extracts from VM-26-resistant cells (25). It seemed reasonable, therefore, to focus on regions of the topoisomerase II sequence that are possible sites of interaction with nucleotides. Phage T4 topoisomerase II is a three-subunit complex, and the gene 39 product provides the ATP-binding and -hydrolysis activity (45). The GyrB subunit of *E. coli* DNA gyrase provides the catalytic site for ATP hydrolysis (46). Alignment of known topoisomerase II amino acid sequences from both prokaryotic and eukaryotic species shows that the gene 39 product and GyrB correspond to the N-terminal third of eukaryotic topoisomerase II (36). Within this ATP domain, three nucleotide-binding consensus sites were identified.

An adenine nucleotide-binding fold described by Walker *et al.* (32) is based on two consensus sequences found in many ATP-binding proteins: the A sequence, GXXGXGKTX₆(I/V), and the B sequence, (R/K)X₂₋₃GX₃L Φ ₂(D/E) (where Φ is a hydrophobic residue). Chin *et al.* (47) have proposed a more general pattern for these two sequences that increases the sensitivity of finding positive ATP-binding sites but does not increase the number of false positives: (G/A)X₄(G/A)(H/K/R)X₀₋₁(T/S/K/R/H) and (H/K/R)X₅₋₈ Φ X Φ ₂(D/E), for the A and B motifs, respectively.

The gene 39 protein of phage T4 has ATPase activity, and residues 125–142 include the conserved GXXGXG motif and roughly conform with the consensus A ATP-binding fold (45). By use of affinity-labeled pyridoxal 5'-diphospho-5'-adenosine, the corresponding region of *E. coli* GyrB has recently been shown to form part of the ATP-binding site (48). The constant GXXGXG pattern of this consensus A sequence, is found at positions 160–165 in the human topoisomerase II protein. A sequence closely matching the modified consensus B structure in *E. coli* GyrB includes residues 413–424 (48). The equivalent amino acids in the human topoisomerase II sequence are at positions 449–460.

A second type of nucleotide-binding site was proposed by Wierenga and Hol (34), based on comparisons of dinucleotide-binding $\beta\alpha$ units in five structurally related enzymes and the human p21 protein. The consensus motif of this dinucleotide-binding $\beta\alpha$ unit has the pattern ϕ X Φ GXXGXGXG₁₂₋₁₅ Φ X Φ X(D,E) (ϕ , hydrophilic residue; Φ , neutral or hydrophobic residue). The invariant GXGXXG motif is also commonly found in the conserved catalytic domain of the protein kinase family (49). Amino acid residues 466–494 of topoisomerase II are homologous to this dinucleotide-binding consensus sequence.

To check for alterations of these nucleotide-binding sites, the sequence of PCR products containing these regions was determined. A DNA fragment of 411 bp that contained the GXXGXG consensus A segment of the ATP-binding fold (32) was produced by PCR using the oligonucleotides OTOPII-5.2 and OTOPII-3.3 with single-strand cDNA as template. PCR products from three separate reactions for each cell line were sequenced directly using OTOPII-5.2 as primer in at least two separate experiments. No sequence change in this region of

FIG. 2. DNA sequence in the region of the base alteration in the VM-26-resistant cells. PCR products were generated from mRNA with primers OTOPII-5.1 and OTOPII-3.1. The resulting 230-bp products were cloned and individually sequenced. A representative sequencing ladder illustrating the G $\rightarrow$ A change in the resistant cells is shown.

the ATP-binding fold was detected in either of the two VM-26-resistant cell lines relative to the sensitive CEM line (data not shown).

Oligonucleotides OTOPII-5.1 and -3.1 were used to produce a 230-bp fragment flanking both the consensus B segment of the ATP-binding fold and the dinucleotide-binding unit in the topoisomerase II sequence. PCR products were cloned into M13mp18 and DNA was isolated from either individual positive plaques or pools of up to 30 positive plaques. The sequences shown in Fig. 2 are examples of individual cloned products. In the drug-resistant cell lines we observed a G $\rightarrow$ A base change at position 1346. Multiple individual cloned PCR products were sequenced and this mutation was seen in five of six CEM/VM-1 sequences, three of four CEM/VM-1-5 sequences, and none of seven CEM sequences. In separate experiments, with the sequence determined from pools of cloned PCR products, the A was always more dominant than the G at position 1346 in both resistant cell lines. The G $\rightarrow$ A change was not detected in any of the cDNA sequences derived from the drug-sensitive cells. This G $\rightarrow$ A shift results in a Arg⁴⁴⁹ $\rightarrow$ Gln substitution (Fig. 3) removing the invariant positively charged amino acid at the start of the consensus B segment of the ATP-binding fold. No changes were found in the sequence of the dinucleotide-binding $\beta\alpha\beta$ unit.

To substantiate this base substitution in the topoisomerase II mRNA sequences, ASOs were synthesized in which the

FIG. 3. Nucleotide and amino acid sequences of the 230-bp fragment containing the G $\rightarrow$ A base substitution. Positions of the oligonucleotide primers used to synthesize the fragment are shown. The sequence corresponding to the consensus B ATP-binding fold (32) is enclosed by broken lines. The region of homology to the consensus sequence for the dinucleotide-binding $\beta\alpha\beta$ unit (34) is enclosed by solid lines. This fragment also contains the topoisomerase II signature sequence (EGDSA), shown enclosed by double lines. The G $\rightarrow$ A substitution is shown at base 1346 with the resulting change of the Arg⁴⁴⁹ to Gln.

FIG. 4. ASO hybridization to PCR products from VM-26-sensitive and -resistant cells. Aliquots of the 230-bp PCR product were spotted on nitrocellulose and hybridized with ASOs. The PCR products were made using cDNA as the template DNA.

center position of the 21-mer was either G (TOPII-ASO1) or A (TOPII-ASO2), complementary to position 1346 of the wild-type or mutant sequence, respectively. Dot blots were prepared using PCR products derived from the cDNA of the sensitive and resistant cells. The mutant TOPII-ASO2 hybridizes only with the PCR product from CEM/VM-1 and CEM/VM-1-5 cDNA, confirming the presence of the mutation (Fig. 4). This alteration is absent in the sensitive CEM cell mRNA. The wild-type TOPII-ASO1 hybridizes to both the sensitive- and the resistant-cell cDNA indicating that both wild-type and mutant alleles are expressed in the resistant cells. This experiment confirms the direct sequencing data showing the presence of the G $\rightarrow$ A substitution only in the VM-26-resistant cells.

One would predict that the domain of the eukaryotic topoisomerase II protein having sequence homology with T4 gene 39 and *E. coli* GyrB protein would contain the ATPase activity. To determine whether any other nucleotide changes existed in the entire region containing the ATPase activity, we used a chemical mismatch cleavage technique that allows single mismatched bases to be identified in relatively large regions of the cDNA fragments (43, 44). Primers OTOPII-5.2 and -3.4 result in the production of a PCR fragment of 1529 bp. This region encompasses amino acids 107–617, extending past the C-terminal end of the T4 gene 39 protein and beyond the point where there is a break in the homology of topoisomerase II with *E. coli* GyrB (36). The only base mismatch detected by this technique was the predicted G $\rightarrow$ A change found previously in the resistant cells. This base substitution results in cleavage yielding a 469-bp fragment (Fig. 5, lanes 4 and 5). The other weak bands in the sample lanes are nonspecific, as they are also present in the unreacted controls (lanes 1 and 2).

DISCUSSION

Topoisomerase II in nuclear extracts from VM-26-resistant CEM cells differs from the enzyme in extracts from sensitive CEM cells in the concentration of ATP required for maximum catalytic activity in the DNA-unknottting assay and in the effect of a nonhydrolyzable analog of ATP, adenosine 5'-[β , γ -imido]triphosphate, on the formation of complexes (20, 25). We have used three independent methods to demonstrate and confirm that a mutant allele for the topoisomerase II gene is expressed in the VM-26-resistant CEM cells. This G $\rightarrow$ A mutation at position 1346 results in mRNA coding for a topoisomerase II protein with glutamine at residue 449 instead of the normal arginine. In other topoisomerase II proteins the analogous position is always arginine or lysine. Fig. 6 shows the homology of the topoisomerase II sequence flanking the alteration site in 10 species (36, 50). Tamura and Gellert (48) recognized that the sequence from 449 to 460 conforms to the consensus B segment of the ATP-binding fold. Arg⁴⁴⁹ is the invariant positively charged

Lane	Cell line	Labeled primer	Chemical treatment
1	CEM	OTOPII-3.4	None
2	CEM	OTOPII-5.2	None
3	CEM	OTOPII-3.4	NH ₂ OH·HCl
4	CEM/VM-1	OTOPII-3.4	NH ₂ OH·HCl
5	CEM/VM-1-5	OTOPII-3.4	NH ₂ OH·HCl
6	CEM	OTOPII-3.4	OsO ₄
7	CEM/VM-1	OTOPII-3.4	OsO ₄
8	CEM/VM-1-5	OTOPII-3.4	OsO ₄
9	CEM/VM-1-5	OTOPII-5.2	NH ₂ OH·HCl
10	CEM/VM-1-5	OTOPII-5.2	OsO ₄

FIG. 5. Chemical mismatch cleavage analysis of topoisomerase II for nucleotide base alterations. Sensitive- and resistant-cell topoisomerase II cDNA was analyzed for base-pair mismatches. The table indicates the cell line from which the cDNA was synthesized, the specific primer that was labeled (5' primer or 3' primer), and the chemical treatment used to detect mismatched bases.

amino acid at the start of the consensus B segment (Fig. 6). The conservation of this positively charged amino acid suggests it may be critical in maintaining the structure or function of the ATP-binding fold.

In the well-studied adenylate kinase protein (47, 51), the A and B consensus segments form a pocket where ATP binds and is hydrolyzed (51, 52). The negatively charged amino acid at the end of the B sequence may serve to bind to the β and γ phosphate of ATP through the chelated Mg^{2+} (52). In topoisomerase II, this amino acid would be Glu⁴⁶⁰, which is

CEM VM-1-5	439	KLDDANDAG-----G	NSTECTLI	ILEGDSA	K	T
Homo sapiens	439	KLDDANDAG-----G	NSTECTLI	ILEGDSA	K	T
D. melanogaster	420	KLDDANEAG-----G	KNSIKCTLI	ILEGDSA	K	S
S. cerevisiae	429	KLEDANKAG-----T	KGYKCTLV	ILEGDSA	S	L
S. pombe	430	KLEDANKAG-----T	KESHKCVLI	ILEGDSA	S	K
T. brucei	417	KLVDATSTR-----R	DPKHRTLI	ILEGDSA	K	A
M. pneumoniae	423	KLADCT-----T	RDPISSELI	IVEGDSA	G	G
E. coli, gyr B	398	ALDLAAGLPGLADQC	ERDPALSEL	YLV	VEGDSA	G
B. subtilis, gyr B	401	ALEISNLPGLADCS	SKDPSISELY	IV	VEGDSA	G
phage T4 gene 39	397	KHIKANLCG-----K	D-ADTTFL	FT	EGDSA	I
phage T2 gene 39	396	KHIKANLCG-----K	D-ADTTFL	FT	EGDSA	I

FIG. 6. Comparison of the region immediately surrounding the mutation site (amino acid 449) in the human topoisomerase II sequence with the corresponding sequences of topoisomerase II from *Drosophila melanogaster*, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Trypanosoma brucei*, and *Mycoplasma pneumoniae*, the DNA gyrase B subunits of *E. coli* and *Bacillus subtilis*, and the gene 39 products of phage T2 and T4 (36, 50). The topoisomerase II signature sequence is boxed. Dashes in the sequences represent gaps introduced for optimal alignment with the bacterial GyrB sequences.

also the first of a five-amino acid signature sequence (EGDSA) that is invariant in all topoisomerase II sequences (Fig. 6).

Single base mutations leading to loss of enzyme activity are well documented. Recent examples include a Gly $\rightarrow$ Glu alteration that results in the expression of an inactive human lipoprotein lipase (53) and an Arg $\rightarrow$ Gly substitution in human interleukin 1 β that reduces biological activity by a factor of 100 (54). Huff *et al.* (55) have developed a *m*-AMSA-resistant strain of phage T4 in which the resistance is the result of a point mutation in the gene 39 product. Denaturing nonequilibrium pH-gradient gel electrophoresis showed an altered mobility for the mutant gene 39 product indicative of a charge alteration in the protein. In *E. coli* GyrB, an Asp⁴²⁶ $\rightarrow$ Asn change in the topoisomerase II signature sequence results in resistance to nalidixic acid (56). The topoisomerase II mutation in our VM-26-resistant cells is analogous to these: a point mutation resulting in a charge alteration in the domain of the topoisomerase II protein that functions in ATP binding and hydrolysis.

Deffie *et al.* (26) reported a mutant topoisomerase II allele in doxorubicin-resistant P388 murine macrophage cells. In these resistant cells there was a reduction in topoisomerase II protein and normal mRNA. It was postulated that in addition to the normal topoisomerase II allele, a shorter mutant allele was present that resulted in the lower levels of full-length topoisomerase II mRNA. It is unknown whether the resistance was secondary to a reduced amount of topoisomerase II or to an alteration in the protein. In the VM-26-resistant cells used in our study, we detected no change in the total amount of immunoreactive topoisomerase II in 1 M NaCl extracts of nuclei (20). However, the amount of topoisomerase II associated with the nuclear matrix is decreased in the VM-26-resistant cells (57).

Cytogenetics of the Drug-Resistant Cells. The gene for human topoisomerase II has been mapped to chromosome 17q21-q22 (5). The CEM parental cells are near triploid ($n = 87$; ref. 58) and the two resistant cell lines have three or four copies of chromosome 17 (M. B. Qumsiyeh, W.T.B., and D.P.S., unpublished observations). The mutation would not be expected to be present in all of the chromosomes 17, which is consistent with expression of both the normal and the mutant topoisomerase II sequence in the CEM/VM-1 and CEM/VM-1-5 cell mRNA. The presence of both sequences was confirmed both by direct sequencing and by hybridization of normal and mutant ASOs (Figs. 2 and 3). Therefore, it is not necessary for the cells to have a complete deficiency of the normal protein to express the resistance phenotype. One hypothesis is that the level of resistance is proportional to the level of the mutant topoisomerase II.

That the VM-26-resistant phenotype is present in cells that express both a normal and mutant allele for topoisomerase II is intriguing from the standpoint of studies involving formation of somatic cell hybrids between the VM-26-resistant and -sensitive CEM cells (59). The IC₅₀ values of three of four hybrid cell lines selected were equal to or only slightly higher than the sensitive parental line, consistent with a recessive phenotype. However, one of the hybrids displayed an IC₅₀ that was >13-fold increased relative to sensitive cells. There was also a reduced level of covalent topoisomerase II-DNA complex formation in one hybrid line. These observations are consistent with our present finding of expression of both normal and altered topoisomerase II mRNA resulting in a drug-resistant phenotype. The increased sensitivity of the hybrid cells could result from an increased ratio of normal to mutant topoisomerase II alleles or from loss of topoisomerase II alleles by chromosome segregation. It will be important to determine whether the mutant allele is present and expressed in the hybrid lines.

Single Allelic Mutation and Homodimer Formation. Topoisomerase II functions as a homodimer (60, 61) and thus is

subject to dominant negative mutations (62). The production of topoisomerase II from both a normal and a mutant allele could result in dimers consisting of either zero, one, or two mutant subunits. A dimer composed of two mutant subunits might have complete loss of function or a decrease in one of the several activities of topoisomerase II. The mutant topoisomerase II subunit in heterodimers could block the normal activity of the protein, thus acting as a dominant negative mutation. In either circumstance expression of a single mutant topoisomerase II allele could result in reduced topoisomerase II activity and the VM-26-resistant phenotype. This type of dominant negative mutation has been documented for the murine growth factor receptor c-Kit (63). Single base changes in the tyrosine kinase domain of c-Kit result in deficiency of the normal kinase activity. Since signal transduction involves oligomers of the receptor, heterodimers of normal and mutant subunits interfere with ligand-induced signal transduction, resulting in fewer active receptors.

Although there is a direct correlation between the Arg⁴⁴⁹ → Gln mutation and the VM-26-resistance phenotype, the precise effect of this mutation on the function of topoisomerase II is unknown. *In vitro* mutagenesis to produce a topoisomerase II protein with Gln⁴⁴⁹ in an appropriate expression vector system would allow characterization of its properties. In addition, analysis of the phenotype of cells transfected with the vector and expressing the altered topoisomerase II should provide direct evidence of the effect of this mutation in altering topoisomerase II activity and resistance to VM-26.

This work was supported by Research Grants CA47941 and CA30103 and by Cancer Center Support Grant CA21765 from the National Cancer Institute; by American Lebanese Syrian Associated Charities; and by a Veterans Affairs Medical Research Merit Review Award.

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