FEBS 26141 FEBS Letters 520 (2002) 161–166

Human small cell lung cancer VPH cells resistant to the bisdioxopiperazine ICRF-187 exhibit a functional dominant Tyr165Ser mutation in the Walker A ATP binding site of topoisomerase IIα

Irene Wessel[a,b], Lars H. Jensen[a,b], Axelle Renodon-Corniere[a,b], Tina K. Sorensen[a,b], John L. Nitiss[c], Peter B. Jensen[b], Maxwell Sehested[a,*]

[a] *Department of Pathology, Laboratory Center, Rigshospitalet 5431, DK-2100 Copenhagen, Denmark*
[b] *Laboratory of Experimental Medical Oncology, The Finsen Center, Rigshospitalet 5074, DK-2100 Copenhagen, Denmark*
[c] *Department of Molecular Pharmacology, St. Jude Children's Research Hospital, Memphis, TN 38101, USA*

Received 4 February 2002; revised 25 April 2002; accepted 26 April 2002

First published online 13 May 2002

Edited by Veli-Pekka Lehto

Abstract Bisdioxopiperazine anti-cancer agents are catalytic inhibitors of topoisomerase II which by unknown means lock the enzyme in a closed clamp form and inhibit its ATPase activity. In order to demarcate a putative pharmacophore, we here describe a novel Tyr165Ser mutation in the enzyme's Walker A ATP binding site leading to specific bisdioxopiperazine resistance when transformed into a temperature-conditional yeast system. The Tyr165Ser mutation differed from a previously described Arg162Gln by being heterozygous and by purified Tyr165Ser enzyme being drug-resistant in a kinetoplast DNA decatenation enzymatic assay. This suggested dominant nature of Tyr165Ser was supported by co-transformation studies in yeast of plasmids carrying wild type and mutant genes. These results enable a model of the bisdioxopiperazine pharmacophore using the proposed asymmetric ATP hydrolysis of the enzyme. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Topoisomerase II; Bisdioxopiperazine; Drug resistance; Mutation

1. Introduction

DNA topoisomerase II is an essential nuclear enzyme that catalyzes the ATP-dependent transport of one DNA double helix through a transient double strand break in a second helix [1,2]. It is required in many aspects of DNA metabolism such as transcription, replication and recombination, as well as being essential for chromosome segregation during mitosis. The mammalian type II enzyme exists as two isozymes, a 180 kDa β enzyme expressed evenly throughout the cell cycle, and a 170 kDa α enzyme that is primarily expressed in the G2/M phase.

Topoisomerase II is one of the most important cellular targets for clinically used anti-cancer drugs [3]. These drugs are divided into two groups according to where they act on the catalytic cycle, namely poisons and catalytic inhibitors. Drugs such as the anthracyclines, the epipodophyllotoxins as well as the aminoacridines all act at the stage in the catalytic cycle where the gate DNA strand is cleaved, stabilizing an enzyme–DNA cleavable complex that leads to irreversible DNA breaks and cell death. These drugs are consequently termed poisons since they convert the essential enzyme into one which damages the cell [4]. Drugs that act on the topoisomerase II catalytic cycle without stabilizing cleavable complexes are termed catalytic inhibitors. The major distinction between poisons and catalytic inhibitors is therefore that the latter do not create DNA breaks at relevant cytotoxic concentrations. In 1991, Ishida et al. [5] described how the bisdioxopiperazine class of anti-cancer agents inhibited the catalytic cycle of topoisomerase II without creating DNA breaks. Subsequent papers from different groups have demonstrated that the bisdioxopiperazines appear to have two highly specific and distinct effects on topoisomerase II, namely locking its N-terminal clamp and inhibiting its ATPase activity [6–8]. The mechanism of action of the bisdioxopiperazine ICRF-193 on yeast topoisomerase II was recently extensively studied by Morris et al. [9] who reported that the drug interacts with the enzyme with one monomer bound to ADP. However, it is still unknown how these drugs molecularly interact with the enzyme. One way of demarcating a possible pharmacophore is to study mutations which confer drug resistance. We and others have previously found such mutations in mammalian cell lines which are clustered in the N-terminal clamp in two sites, namely at Thr49Ile [10] and Tyr50Phe [11], and at Arg162Gln [12] in the Walker A ATP binding motif of aa ^{161}GXXGXG^{166} [13]. In addition, using random mutagenesis and selection in yeast, two groups independently discovered a Leu169Phe mutation which is just next to this Walker A motif [14,15]. In the present study we describe a novel Tyr165Ser mutation that was detected in human small cell lung cancer cells which differs from the neighboring Arg162Gln in several respects. Using the asymmetric ATP hydrolysis of topoisomerase II blocked by ICRF-193 proposed in [9], this enables a model of the putative bisdioxopiperazine pharmacophore to be put forward.

2. Materials and methods

2.1. Drugs

The bisdioxopiperazine ICRF-187 (dexrazoxane, Cardioxane®) was commercially obtained from Chiron (Amsterdam, The Netherlands), vincristine from Lilly, (Copenhagen, Denmark), aclarubicin from

*Corresponding author. Fax: (45)-3554-5414.
E-mail address: mxs@rh.dk (M. Sehested).

Abbreviations: kDNA, kinetoplast DNA; wt, wild type

0014-5793/02/$22.00 © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.
PII: S0014-5793(02)02805-3

Lundbeck (Copenhagen, Denmark), etoposide and cisplatin from Bristol-Myers Squibb (Lyngby, Denmark) and camptothecin from Sigma (Kansas City, MO, USA). Merbarone was a generous gift from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment, NCI, NIH, Bethesda, MD, USA.

2.2. Cells and clonogenic assay

Human small cell lung cancer NYH cells were selected through approximately 20 passages in increasing concentrations of ICRF-187 until they were resistant to 1 mM drug. A 3 week clonogenic assay using soft agar on a sheep red blood cell feeder layer was used with continuous drug incubation.

2.3. Alkaline elution assay

The alkaline elution assay for measuring DNA single strand breaks in intact NYH cells was performed as described in [11].

2.4. Western blot and band depletion assays

Western blots were performed as previously described in [12] using either a monoclonal antibody for the α (Cambridge Research Biochemicals, Cheshire, UK) or a polyclonal antibody for the β isoform (Bio-Trend, Cologne, Germany). Band depletion assays are Western blots performed on nuclear extracts following drug incubation of viable cells for 1 h [12].

2.5. Sequencing of topoisomerase IIα

RT-PCR and PCR were performed as previously described [16]. Sequencing of the PCR products was performed using the ABI Prism Dye Terminator Cycle Sequencing kit (Perkin Elmer) from both 5′ and 3′ ends for two or three times each on two different mRNA extractions using the primers previously described in [16].

2.6. Long PCR and restriction cleavage

In order to determine the expression of wild type (wt) and mutant alleles, a full-length PCR was performed with the TaqPlus Long PCR System (Stratagene, Amsterdam, The Netherlands) using the primers: Topstart2: 5′-CCT GTT TAG TCG CTT TCA GGG TTC TTG AGC-3′ (−48 to −19) and Stop2: 5′-GGA AGT TAA GAG CTT CAG GTA AC-3′ (4675–4653). The Tyr165Ser mutation creates an additional restriction site for *Bpm*I (New England Biolabs, Frankfurt am Main, Germany) leading to two restriction sites in its gene, while the wt only has one.

2.7. Functional characterization of mutant human topoisomerase IIα in yeast

To assess whether the Tyr165Ser mutation found in NYH/187/165 topoisomerase IIα contributes to the acquired bisdioxopiperazine resistance, the corresponding point mutation was introduced into the episomal expression vector for human topoisomerase IIα pMJ1 by oligonucleotide-directed mutagenesis as previously described [12] using the two mutagenic primers Tyr165Ser-SN: GGT CGA AAT GGC TCT GGA GCC AAA TTG TG and Tyr165Ser-ASN: CAC AAT TTG GCT CCA GAG CCA TTT CGA CC. In addition, the Arg450Gln mutation which has been reported to confer resistance to topoisomerase II poisons was also introduced using the primers described in [17].

2.8. Purification and testing of wt and mutant human topoisomerase IIα

Human topoisomerase IIα was expressed for purification using the plasmid YEpWOB6 [18] in the protease-deficient yeast strain JelΔ TOP1 (*trp1, leu2, ura-52, pbr1-1122, pep4-3, Δhis3::PGAL10-GAL4, TOP1::LEU2*). Induction of topoisomerase II by galactose and protein purification were as described in [12].

2.8.1. Decatenation assay. Topoisomerase II catalytic activity was measured by kinetoplast DNA (kDNA) decatenation using ^{3}H-labeled kDNA isolated from *Crithidia fasciculata* (ATCC, Rockville, MD, USA) as described in [12]. Briefly, purified wt or Y165S mutant topoisomerase IIα was incubated with 0.2 μg kDNA for 15 min at 37°C in a final volume of 20 μl in a buffer containing 50 mM Tris–HCl, pH 8, 120 mM KCl, 10 mM $MgCl_2$, 1.0 mM ATP, 0.5 mM dithiothreitol, 30 μg bovine serum albumin per milliliter). After addition of stop buffer/loading dye mix (5% sarcosyl, 0.0025% bromophenol blue, 25% glycerol), samples were loaded on 1% agarose/0.5% ethidium bromide gels and run in TBE buffer containing 0.5 μg/ml ethidium bromide at 100 V for approximately 50 min. Loading wells were cut out and scintillation counted. Thus, full activity of the enzyme entails no remaining kDNA in the well leading to a cpm of around 0, while inhibition of the enzyme by drug leads to increasing cpm values (see Fig. 3).

2.8.2. DNA cleavage assay. Determination of covalent complexes between purified wt or mutated topoisomerase II and radiolabelled DNA induced in vitro by a drug was as described in [19].

2.9. Coexpression experiments

The constructs pMJ1 and pKN9 are both single copy episomal ARS/CEN-based yeast expression vectors for human topoisomerase IIα which express the protein from the constitutive and cell cycle-independent yeast topoisomerase I promoter. pMJ1 is based on yCP50 and carries a URA3 selectable marker while pKN9 is based on pLAC111 and carries a LEU2 yeast selectable marker. These constructs have been described in detail elsewhere [17,19]. In order to compare the dominance of the identified Tyr165Ser mutation with the previously described Tyr50Phe mutation, co-expression experiments were performed using the drug-permeable yeast strain JN362At2-4 (*MATa, ura5-2, leu2, trp1, his7, ade1-2, ISE2, leu2, top2-4*) which carries a temperature-sensitive chromosomal t2-4 topoisomerase II allele which is inactive at 34°C. The following co-transformations were performed: (i) pMJ1-WT (URA3)+pLAC111 (LEU2), (ii) pMJ1-Tyr50Phe (URA3)+pLAC111 (LEU2), (iii) pMJ1-Tyr165Ser (URA3)+pLAC111 (LEU2), (iv) pMJ1-WT (URA3)+pKN9 (LEU2), (v) pMJ1-Tyr50Phe (URA3)+pKN9 (LEU2), (vi) pMJ1-Tyr165Ser (URA3)+pKN9 (LEU2). Propagation of transformed cells was carried out on SC-LEU-URA medium to ensure selection for both plasmids as described in [19]. Clonogenic assays to determine cytotoxicity involving yeast cells containing pLAC111 (topoisomerase IIα expressed alone) were repeated two or three times, while assays involving cells containing pKN9 (co-expression of topoisomerase IIα) were repeated three to five times.

3. Results

3.1. Cells and drug resistance

Three subcultures of NYH cells developed resistance to ICRF-187 while maintaining unaltered morphology and doubling times. Sequencing of the entire topoisomerase IIα cDNA from these three sublines showed a *homozygous* G→A point mutation at nt 485 as previously described [12] in one case (designated NYH/187/162), while the other two sublines revealed identical *heterozygous* A→C point mutations at nt 494 causing a Tyr165Ser conversion which is also in the Walker A motif (designated NYH/187/165). As shown in Table 1, NYH/187/165 cells are highly resistant to the selecting drug, ICRF-187, while demonstrating only minor changes in sensitivity to other drug classes. To assess whether the newly found Tyr165Ser mutation confers bisdioxopiperazine resistance, the corresponding point mutation was introduced into the episomal expression vector for human topoisomerase IIα pMJ1 by oligonucleotide-directed mutagenesis. Clonogenic assay in yeast revealed a highly specific resistance to both ICRF-187 and ICRF-193, but no cross-resistance to either of the topoisomerase II poisons etoposide or m-AMSA (Fig. 1). The Arg450Gln mutation in the Walker B consensus site of human topoisomerase IIα has been reported to confer resistance to topoisomerase II poisons [17]. Further, in its purified form it has been shown to exhibit impaired ATP utilization [20] as is also seen in the Arg162Gln [12] and the Tyr165Ser (see below) mutants. We were therefore interested to see whether the Arg450Gln conferred resistance to bisdioxopiperazines. As shown in Fig. 2, there is no cross-resistance to ICRF-187 in yeast cells transformed with human Arg450Gln topoisomerase IIα. This indicates that it is not the increase in ATP requirement that is responsible for resis-

Table 1
Sensitivity of various anti-cancer drug classes in NYH and NYH/187/165 cells

Drug (μM)	NYH	NYH/187/165	RR
Camptothecin	0.0015 (0.001)	0.0027 (0.000)	1.8
Etoposide	0.0407 (0.001)	0.1363 (0.001)	3.4
Amsacrine	0.027 (0.000)	0.0529 (0.003)	2
Cisplatin	0.3363 (0.176)	0.3163 (0.225)	0.9
Merbarone	4.6603 (0.806)	4.6013 (1.066)	1
Aclarubicin	0.0021 (0.001)	0.0025 (0001)	1.2
Vincristine	0.0013 (0.000)	0.0013 (0.000)	1
ICRF-187	17.885 (3.019)	∞	∞

High level resistance is only observed to the selecting agent. S.D.s of three determinations are in parentheses. RR = relative resistance.

tance to the bisdioxopiperazines in the Arg162Gln and Tyr165Ser mutants.

3.2. Similarities and differences between Arg162Gln and Tyr165Ser

As both Arg162 and Tyr165 are in the Walker A ATP binding site, we have compared the differential phenotypes of these mutations to obtain a better understanding of the putative pharmacophore. A summary is also supplied in Table 2.

3.3. Similarities

In addition to the specificity of the bisdioxopiperazine resistance described above, the following assays showed a similar result in NYH/187/162 and/165 cells: protection of etoposide-induced toxicity by ICRF-187 was reduced by approximately 50% in NYH/187/165 cells in a clonogenic assay, and inhibition of etoposide-induced DNA single strand breaks by ICRF-187 measured by the standard alkaline elution technique was abolished in NYH/187/165 cells (not shown). These results are similar to those reported for NYH/187/162 cells [12]. Further, using Western blotting the topoisomerase IIα and β levels were equal in NYH and NYH/

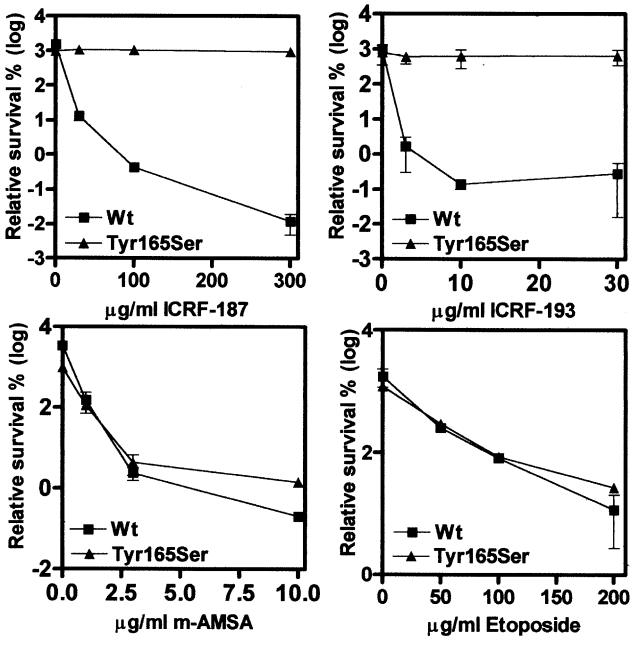

Fig. 1. Clonogenic assay in yeast cells transformed with either wt or Tyr165Ser human topoisomerase IIα and incubated in increasing concentration of drugs as indicated. Yeast cells with Tyr165Ser topoisomerase IIα are highly resistant to both ICRF-187 and -193 (upper panels), but show comparable sensitivity to the typical topoisomerase II poisons, m-AMSA and etoposide (lower panels).

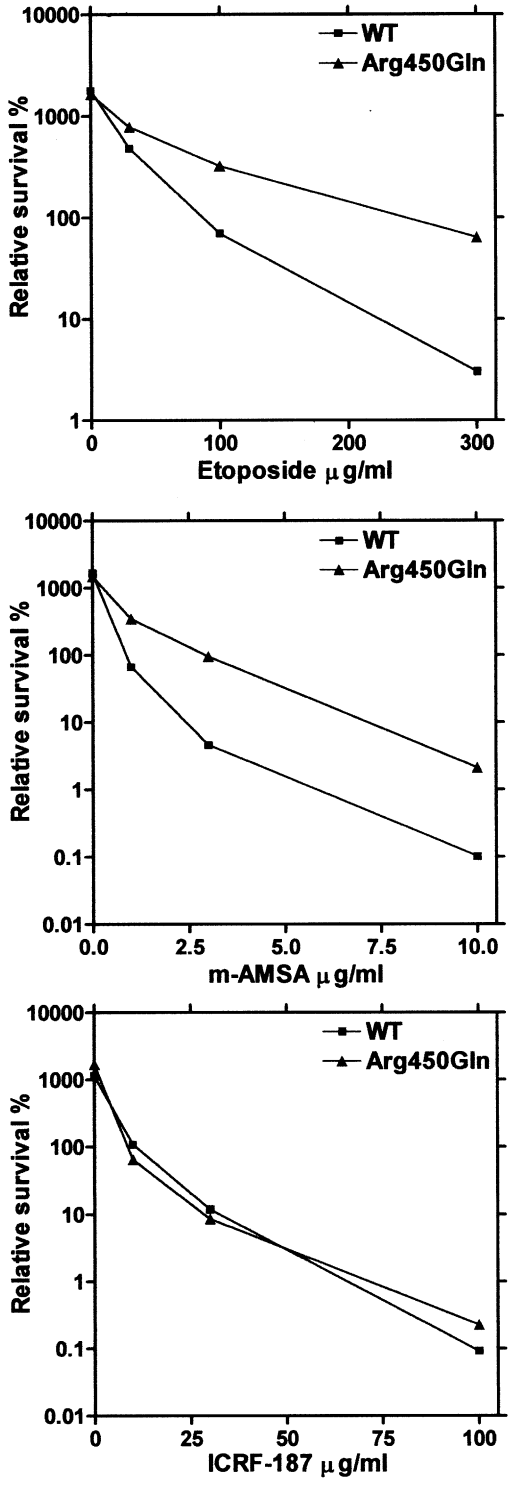

Fig. 2. Clonogenic assay in yeast cells transformed with either wt or Arg450Gln human topoisomerase IIα and incubated in increasing concentration of drugs as indicated. Yeast cells with Arg450Gln topoisomerase IIα exhibit the expected resistance to both etoposide (top panel) and m-AMSA (middle panel), but have the same sensitivity to ICRF-187 (lower panel).

Table 2
Summary of the similarities and differences between wt and the three functional mutations derived from mammalian cell lines selected for bisdioxopiperazine resistance

Mutation	wt	Tyr50Phe	Arg162Gln	Tyr165Ser
Topo IIα band depl. by ICRF-187	yes	no	no	no
Inhib. of etoposide-induced SSBs	yes	no	no	no
Inhib. of etoposide cytotox. by ICRF	yes	no	yes	yes
DNA cleavage at high ICRF-187 concentration	yes	ND	no	no
Increase in ATP demand	wt	ND	yes	yes
ICRF-187 inhibition of kDNA decatenation	sensitive	resistant	sensitive	resistant
Dominance/recessiveness	wt	rec	rec	dom

Abbreviations: dom, dominant; ND, not done; rec, recessive; SSB, single strand breaks; topo, topoisomerase.

187/165 cells. Following 1 h incubation with ICRF-187, a decrease in topoisomerase IIβ was observed in extracts from both NYH and NYH/187/165 cells, while the α isoform was only depleted in NYH cells and thus unchanged in NYH/187/165 cells indicating a specificity for the α isoform (not shown). The results using Western blotting are thus the same as in NYH/187/162 cells [12]. Though bisdioxopiperazines do not exert their cytotoxicity by cleavable complex formation, they are able to create a small number of DNA breaks at very high concentrations [19]. Utilizing a conventional DNA cleavage assay, ICRF-187-induced cleavable complexes were completely lacking in both Arg162Gln and Tyr165Ser enzymes, while wt enzyme did produce low level cleavage (not shown). Finally, the ATP concentration needed for 50% catalytic activity in the kDNA decatenation assay was ~30 μM for purified wt enzyme while it was ~140 μM for purified Tyr165Ser enzyme (not shown). This increase in ATP demand in the Tyr165Ser enzyme is similar to that reported for Arg162Gln [12].

3.4. Differences

The two distinct differences between the Arg162Gln and the Tyr165Ser mutations lie in the ability of ICRF-187 to inhibit the catalytic activity of the enzyme using the decatenation assay and in the suggested dominant nature of the Tyr165Ser mutation. In the catalytic kDNA decatenation assay, purified Tyr165Ser topoisomerase IIα was completely resistant to ICRF-187 while Arg162Gln was sensitive (Fig. 3). This increased phenotype of Tyr165Ser was also observed while sequencing the cDNA as NYH/187/162 cells showed a *homozygous* G→A point mutation at nt 485, while NYH/187/165 had a *heterozygous* A→C point mutation at nt 494. This heterozygosity was confirmed using the restriction enzyme *Bpm*I which cuts only once in the full-length wt gene, while the nt 494 A→C mutation itself introduces an additional restriction site enabling both transcripts to be evaluated. Utilizing *Bpm*I on a long topoisomerase IIα PCR from NYH/187/165 revealed a cDNA with equally represented 494A and 494C transcripts demonstrating that both alleles are actively transcribed (Fig. 4). In order to explore the possibility of the Tyr165Ser mutation being dominant, we co-expressed either Tyr50Phe or Tyr165Ser human topoisomerase IIα in yeast cells which also expressed wt human topoisomerase IIα protein. The Tyr50Phe mutant was used as it shows the same resistance to ICRF-187 as Tyr165Ser in the decatenation assays [11], while Arg162Gln is drug-sensitive in this assay (Fig. 3). Single expression of either Tyr50Phe or Tyr165Ser conferred, as expected, high level drug resistance to ICRF-187 in a 24 h clonogenic assay (Fig. 5, upper panel). However, when coexpressed with wt, it is obvious that the increase in sensitivity in wt/Tyr50Phe is much greater than that conferred by wt/

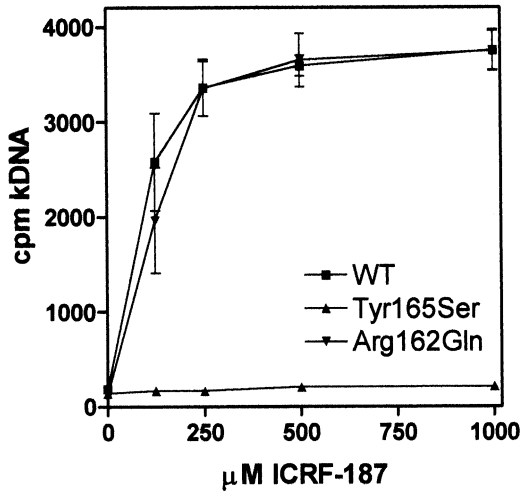

Fig. 3. Catalytic topoisomerase II kDNA decatenation assay using purified human wt and Tyr165Ser and Arg162Gln mutant enzyme. Tyr165Ser enzyme is completely resistant to ICRF-187-mediated inhibition of decatenation while Arg162Gln demonstrates wt sensitivity.

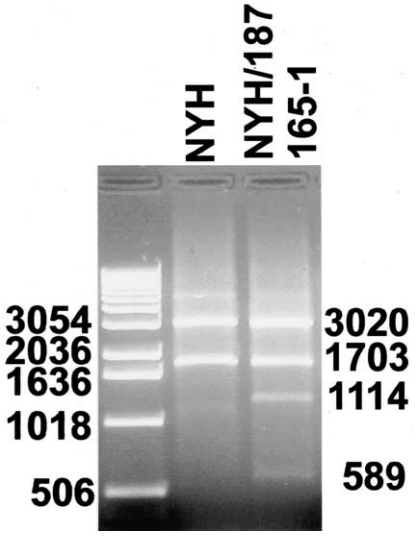

Fig. 4. Full-length cDNA products (4723 nt) of wt NYH and NYH/187/165 digested with *Bpm*I demonstrating a heterozygous expression of wt and mutant topoisomerase IIα cDNA in NYH/187/165 cells. Thus, wt NYH cDNA has one restriction site creating two bands of 3020 and 1703 nt, while the A→C point mutation at nt 494 in NYH/187/165 cDNA creates a new restriction site that cleaves the 1703 nt fragment into 1114 and 589 nt bands.

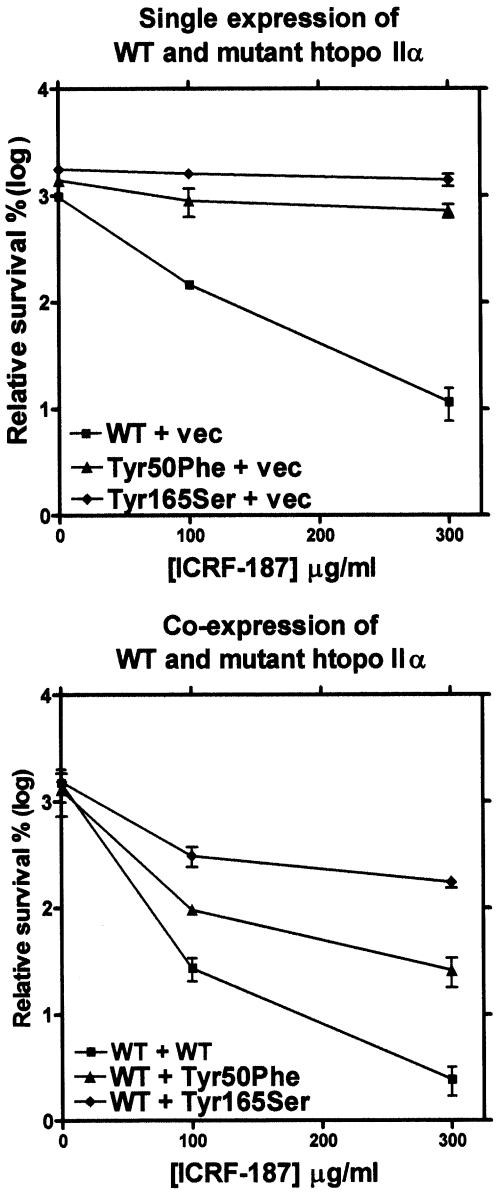

Fig. 5. Clonogenic assay in yeast cells overexpressing either wt, Tyr50Phe or Tyr165Ser human topoisomerase IIα together with an empty vector (upper panel) or co-expressed with wt human topoisomerase IIα (lower panel). Both Tyr50Phe and Tyr165Ser mutant proteins display high level resistance towards bisdioxopiperazines when co-expressed with the empty vector. However, when co-expressed with wt protein the Tyr50Phe transfected cells become approximately a log more sensitive to ICRF-187 than wt/Tyr165Ser cells.

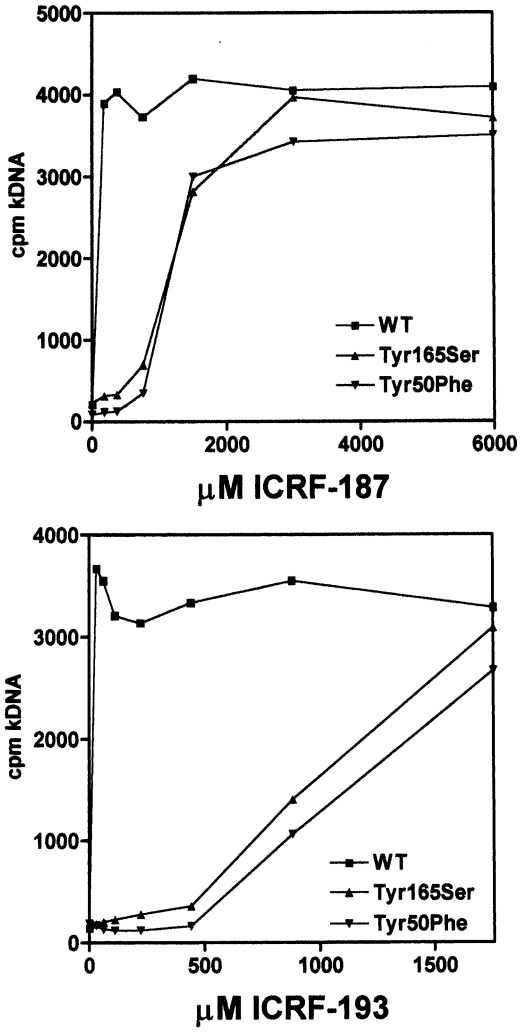

Fig. 6. Comparison of inhibition of purified human wt and Tyr165Ser and Tyr50Phe at very high concentrations of ICRF-187 (upper panel) and ICRF-193 (lower panel). Both mutant enzymes are inhibited to a similar degree indicating that their intrinsic drug resistance as homodimers is the same.

Tyr165Ser (Fig. 5, lower panel). As topoisomerase II is a dimeric enzyme, this indicates that wt/Tyr50Phe heterodimers are more drug-sensitive than wt/Tyr165Ser heterodimers.

4. Discussion

Bisdioxopiperazines have a dual effect on topoisomerase II, namely locking its N-terminal clamp and inhibiting its ATPase activity. Olland and Wang [8], using the N-terminal 1–409 aa of yeast topoisomerase II, showed that the bisdioxopiperazine ICRF-193 inhibited the ATPase activity of this fragment suggesting that the drug interacts directly with the paired N-terminal clamp. There is thus an indication of a bisdioxopiperazine pharmacophore in the N-terminal clamp. On the other hand, it is conceivable that the mutations in and around the Walker A ATP binding site confer bisdioxopiperazine resistance in an indirect way by shifting the equilibrium of the enzyme to the early, open clamp form which would obviously hinder the bisdioxopiperazine to lock the enzyme in its closed clamp configuration. This could be achieved by an increase in their ATP requirement, which is indeed found in both Arg162Gln and Tyr165Ser mutant enzymes. However, as shown in Fig. 2, yeast cells carrying the Arg450Gln mutation in the Walker B ATP binding site, which also has an increased ATP requirement, lack resistance to ICRF-187, indicating that changes in ATP requirement per se are not sufficient. Thus, the question is whether the Walker A ATP binding site is part of the bisdioxopiperazine pharmacophore. In this respect, the new Tyr165Ser mutation is of interest for several reasons. Firstly, it obviously confirms and enhances the importance of the Walker A motif in bisdioxopiperazine resistance. It is also the first mutation to be expressed in a heterozygous fashion which led us to study this phenomenon in

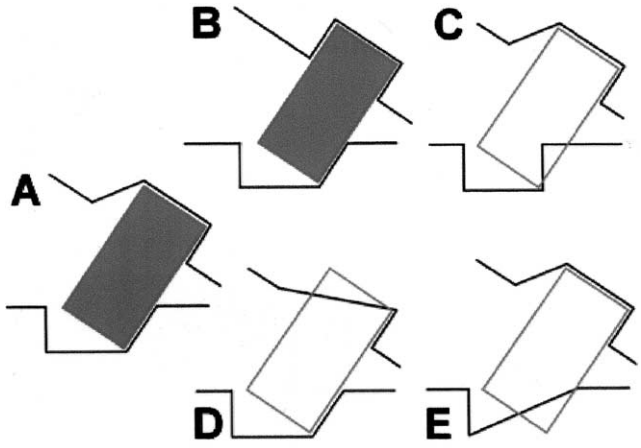

Fig. 7. A model for bisdioxopiperazine drug binding to the N-terminal domain of topoisomerase IIα. The drug binds to the stage of the enzyme's catalytic cycle where one ADP is bound while the other ATP binding site is free, indicating an asymmetric state. The drug is illustrated by a solid rectangle when drug binding is possible, and by a transparent one when drug binding is unattainable. A: Binding of drug to wt enzyme. B,C: Models of recessive mutations illustrating that in order to accomplish complete resistance both monomers need to be mutated. D,E: Models for dominant mutations illustrating that one mutated monomer is sufficient to confer complete resistance.

more detail. As shown in Fig. 5, the wt/Tyr50Phe transformant is more drug-sensitive than wt/Tyr165Ser. In addition, in order to exclude the possibility of the Tyr165Ser enzyme in itself being more drug-resistant, we compared it with purified Tyr50Phe at very high concentrations of both ICRF-187 and its more potent analogue, ICRF-193. As shown in Fig. 6, there was no difference between the mutants, again suggesting that the difference in sensitivity in the wt/mutant co-expression shown in Fig. 5 is due to dominance in the Tyr165Ser mutant compared to recessiveness in Tyr50Phe. Thus, while not biochemically proven, the combined results in this paper indicate that Tyr165Ser protein is dominant while Tyr50Phe and Arg162Gln are recessive. If so, it is thus interesting that while the loss of charge in the Arg162Gln mutation and loss of a hydroxyl group in the Tyr50Phe appear to have to be present in both dimers to exert their effect on drug sensitivity, the structurally more radical loss of a benzyl moiety in Tyr165Ser appears to be sufficient to cause resistance by being in just one of the dimers. As recently shown in a detailed kinetic study [9], the bisdioxopiperazine ICRF-193 is deduced to interact with topoisomerase II bound to one ADP with the other ATP site open, and actually allowing ATP hydrolysis at this other site. The result of this complex picture is that ICRF-193 is not a competitive inhibitor of ATP, and that topoisomerase II bound to drug continues to hydrolyze ATP at a reduced rate [9]. This indicates that drug binding requires a strict steric and/or even asymmetric conformation of the enzyme. Theoretically the mutations, which are all located in the area between the γ-phosphates, could interfere either with this asymmetry, or with a putative drug binding pocket. Assuming that the drug acts on the asymmetric state of the enzyme, one would suppose that there is only one binding site per enzyme dimer. If so, this could account for the differences found in relation to the localization of the mutants and of the recessive/dominant role of the mutants as well. A suggested model for drug binding based on these assumptions is shown in Fig. 7.

Acknowledgements: The expert technical assistance of Sanne Christiansen and Annette Nielsen is highly appreciated. The work was supported by the Danish Cancer Society, The H:S Research Council, The Novo Foundation, The National Cancer Institute (USA) CA52814, Core Grant CA21765 and the American Lebanese Syrian Associated Charities (ALSAC).

References

[1] Wang, J.C. (1998) Q. Rev. Biophys. 31, 107–144.
[2] Berger, J.M., Gamblin, S.J., Harrison, S.C. and Wang, J.C. (1996) Nature 379, 225–232.
[3] Chen, A.Y. and Liu, L.F. (1994) Annu. Rev. Pharmacol. Toxicol. 34, 191–218.
[4] Wasserman, R.A. and Wang, J.C. (1994) Cancer Res. 54, 1795–1800.
[5] Ishida, R., Miki, T., Narita, T., Yui, R., Sato, M., Utsumi, K.R., Tanabe, K. and Andoh, T. (1991) Cancer Res. 51, 4909–4916.
[6] Roca, J., Ishida, R., Berger, J.M., Andoh, T. and Wang, J.C. (1994) Proc. Natl. Acad. Sci. USA 91, 1781–1785.
[7] Chang, S., Hu, T. and Hsieh, T.S. (1998) J. Biol. Chem. 273, 19822–19828.
[8] Olland, S. and Wang, J.C. (1999) J. Biol. Chem. 274, 21688–21694.
[9] Morris, S.K., Baird, C.L. and Lindsley, J.E. (2000) J. Biol. Chem. 275, 2613–2618.
[10] Yalowich, J.C., Thampatty, P., Allan, W.P., Chee, G. and Hasinoff, B. (1998) Proc. Am. Assoc. Cancer Res. 39, 375.
[11] Sehested, M., Wessel, I., Jensen, L.H., Holm, B., Oliveri, R.S., Kenwrick, S., Creighton, A.M., Nitiss, J.L. and Jensen, P.B. (1998) Cancer Res. 58, 1460–1468.
[12] Wessel, I., Jensen, L.H., Jensen, P.B., Falck, J., Rose, A., Roerth, M., Nitiss, J.L. and Sehested, M. (1999) Cancer Res. 59, 3442–3450.
[13] Walker, J.E., Saraste, M., Runswick, M.J. and Gay, N.J. (1982) EMBO J. 1, 945–951.
[14] Jensen, L.H., Wessel, I., Møller, M., Nitiss, J.L., Sehested, M. and Jensen, P.B. (2000) FEBS Lett. 480, 201–207.
[15] Patel, S., Jazrawi, E., Creighton, A.M., Austin, C.A. and Fisher, L.M. (2000) Mol. Pharmacol. 58, 560–568.
[16] Wessel, I., Jensen, P.B., Falck, J., Mirski, S.E., Cole, S.P. and Sehested, M. (1997) Cancer Res. 57, 4451–4454.
[17] Hsiung, Y., Jannatipour, M., Rose, A., McMahon, J., Duncan, D. and Nitiss, J.L. (1996) Cancer Res. 56, 91–99.
[18] Wasserman, R.A., Austin, C.A., Fisher, L.M. and Wang, J.C. (1993) Cancer Res. 53, 3591–3596.
[19] Jensen, L.H., Nitiss, K.C., Rose, A., Dong, J., Zhou, J., Hu, T., Osheroff, N., Jensen, P.B., Sehested, M. and Nitiss, J.L. (2000) J. Biol. Chem. 275, 2137–2146.
[20] Mao, Y., Yu, C., Hsieh, T.S., Nitiss, J.L., Liu, A.A., Wang, H. and Liu, L.F. (1999) Biochemistry 38, 10793–10800.