isomerase II activity in the nuclear extract and cell survival increased with increasing time after drug removal (data not shown). Novobiocin inhibits eukaryotic topoisomerase II by acting as a competitive inhibitor of ATP, which is required for enzyme activity [5]. It does not damage DNA under the conditions used in our experiments and is regarded as essentially nontoxic for eukaryotic cells. Hypersensitivity of Raji-HN2 (and, perhaps, other resistant tumor) cells to novobiocin is correlated with the inhibition of elevated topoisomerase II activity of these cells. A change in the ATP-binding site of the enzyme may produce an enormous increase in the susceptibility of the enzyme to the inhibitory binding of novobiocin.

The differential sensitivity to novobiocin exhibited by Raji and Raji-HN2 cells provides a clinical rationale for the use of novobiocin, or a novobiocin-like compound, in cancer chemotherapy. Novobiocin can be used under two different clinical settings. In the first instance, in view of the synergistic action of novobiocin when used with HN2 for Raji cells, the use of low concentrations of novobiocin in combination with HN2, or other alkylating agents, could selectively increase the destruction of sensitive tumor cells. Second, the use of novobiocin alone, in high concentrations, could selectively kill tumors that have developed resistance to alkylating agents, and which have elevated topoisomerase II activity. Novobiocin itself would likely have no effect on nonmalignant cells or on the HN2-sensitive fraction.

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Altered expression and transcription of the topoisomerase II gene in nitrogen mustard-resistant human cells

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Topoisomerase II, a target of several classes of anti-tumor drugs, is involved in chromosome segregation and is implicated in gene regulation, structure and function [see reviews in Refs. 1–3]. Recently we reported that Raji-HN2, a Burkitt lymphoma cell line made resistant to HN2*, is hypersensitive to topoisomerase II inhibitors and contains about 3-fold more of extractable topoisomerase II activity than the parental Raji cell line [4, 5]. Although the function of topoisomerase II in Raji-HN2 cells is unknown, the increased extractable enzyme activity in these cells is correlated with resistance to HN2 and increased cell doubling time [5]. In this paper we report, using antiserum and cDNA specific for topoisomerase II as probes, that the synthesis of the enzyme and transcription of the gene were elevated in Raji-HN2 cells when compared with Raji cells.

Materials and methods

Cells. Raji-HN2 cells were treated weekly with 10 μM HN2 to maintain the resistant phenotype [5, 6]. In the experiments described below, both Raji and Raji-HN2 cells were harvested simultaneously at the exponential growth phase.

Protein analysis. Cells were lysed directly in SDS buffer and electrophoresed in a 7.5% acrylamide gel [7]. The fractionated polypeptides were blotted to a nitrocellulose membrane and reacted sequentially with a polyclonal antiserum to purified calf thymus topoisomerase II and ^{125}I-labeled protein A [8].

RNA analysis. RNA prepared from cells lysed with guanidine isothiocyanate was fractionated on an oligo-dT column to yield poly(A) RNA [9]. After electrophoresis in a 1% agarose gel containing 1 M formaldehyde [9], the RNA was blotted directly to a Zetaprobe nylon membrane (Bio-Rad) with 0.2 M NaOH for 1.5 hr. The blot was then neutralized with 2 × SSPE for 10 min.

DNA analysis. DNA extracted from nuclei [9] was digested with restriction enzymes (2 units/μg DNA for 6 hr at 37°), fractionated in a 1% agarose gel, and transferred to a Zetaprobe membrane by blotting with 0.4 M NaOH [10].

cDNA cloning. A Raji-HN2 cDNA library was prepared by linker-ligation at the EcoRI site of bacteriophage λgt11 [11]. The library (2 × 10^5 plaques) was screened, under low stringency conditions [9], with a *Drosophila* topoisomerase II cDNA clone [12], and twenty-six positive clones were obtained. Two partial clones, SP-12 and SP-17, were used in this study.

* Abbreviations: HN2, nitrogen mustard [2-chloro-*N*-(2-chlorethyl)-*N*-methylethanamine]; cDNA, complementary DNA; kb, kilobase; kD, kilodalton; SDS, sodium dodecyl sulfate; and 1 × SSPE = 180 mM NaCl, 10 mM $NaPO_4$, pH 7.7, 1 mM EDTA.

Results and discussion

Raji-HN2 cells contain approximately three times more extractable topoisomerase II activity than Raji cells [5]. To determine whether this increased enzyme activity represents increased specific activity or increased enzyme amount, the cells were analysed by immunoblotting techniques. Two proteins (170 kD and 180 kD) in both cell lines reacted with topoisomerase II antiserum (Fig. 1). Raji cells contained predominantly the 170 kD protein. Raji-HN2 cells contained about the same amount of the 170 kD protein as Raji cells, but the amount of the 180 kD protein was increased markedly (Fig. 1). Raji-HN2 cells are hypersensitive to topoisomerase II inhibitors [5], and this hypersensitivity can be correlated with the increased content of the 180 kD topoisomerase II. The 170 kD and 180 kD forms of topoisomerase II have been purified from murine cells and were found to be distinct enzymes that share common peptide sequences [7].

We cloned the human topoisomerase II cDNAs and used them as probes to study the transcription of this gene. The complete nucleotide sequence of a partial 3′-cDNA clone, SP-17 (see Fig. 2), was determined, and the data predict that the human topoisomerase II is 74% homologous to the *Drosophila* enzyme at the protein level*. SP-12 hybridized to Raji topoisomerase II genomic sequences that were 5′ to those hybridizing to SP-17.*

SP-17 hybridized to two poly(A) RNA species extracted from Raji-HN2 and Raji cells (Fig. 2). The larger RNA (6 kb) was of the approximate size to encode a protein of 170–180 kD. The RNA extracted from Raji-HN2 cells routinely migrated slower than that extracted from Raji cells (Fig. 2). Because of the small difference in size between the 170 kD and 180 kD topoisomerase II, their transcripts were not resolved in the gel system used. We quantitated the 6 kb RNA by comparing it with β2-microglobulin mRNA, which was present in equivalent amounts in both Raji and Raji-HN2 cells (Fig. 2). We found, in two separate experiments, that Raji-HN2 cells had 2.1 and 2.8 times more of the 6 kb RNA than Raji cells. This result is in good agreement with those obtained with antiserum (Fig. 1) and enzyme activity assay [5]. Thus, increased transcription resulted in an increased content of topoisomerase II in Raji-HN2 cells.

SP-17, but not SP-12, also hybridized to a 2 kb RNA (Fig. 2). The nature of this RNA, which was also present in other human cells (data not shown), is unknown. It may be a breakdown product of the 6 kb RNA, or it may be a transcript that encodes a novel topoisomerase II. The sequences specific for the 2 kb RNA were mapped to the distal 3′ end of SP-17.* RNA lacking poly(A) sequences did not hybridize to topoisomerase II cDNA (Fig. 2).

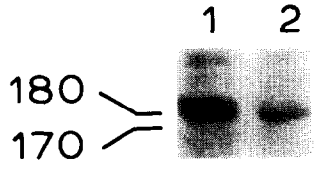

Fig. 1. Immunoblot of topoisomerase II. Raji-HN2 (1) or Raji (2) cells (1×10^6) were lysed and analyzed by immunoblotting. The molecular masses (kD) of the two immunoreactive proteins are indicated.

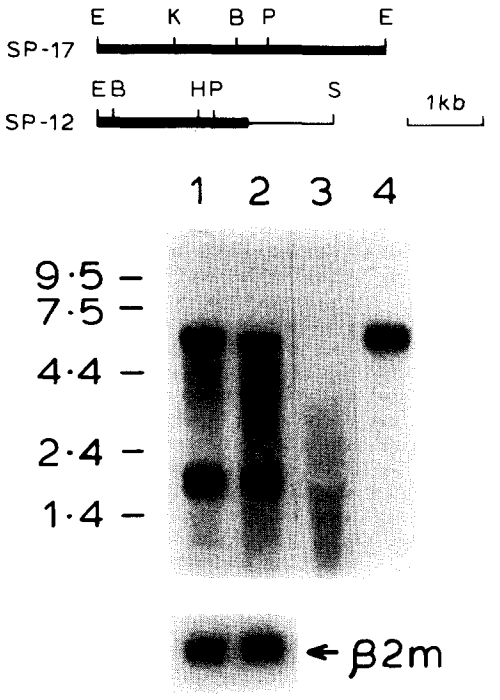

Fig. 2. Northern blot analysis of topoisomerase II transcripts. Upper panel: Restriction maps of human topoisomerase II cDNA. One of the Eco RI sites of SP-12 was lost and the insert was rescued by digestion with Eco RI and Sst I. Thick line = cDNA sequences; thin line = vector sequences. Enzymes: Bam HI (B), Eco RI (E), Hind III (H), Kpn I (K), Pst I (P), and Sst I (S). Lower panel: Topoisomerase II specific RNA. Northern blots of 2 μg of poly(A) RNA or 25 μg of RNA lacking poly(A) were hybridized to [^{32}P]nick-translated SP-12 or SP-17 inserts at 42° for 48 hr. Hybridization buffer: 5 × SSPE, 33% formamide, 200 μg/ml heparin, 0.2% SDS, 0.2% sodium pyrophosphate and 6% dextran sulfate. The blots were washed under high stringency conditions [9]. The hybridized probe was stripped from the blot with boiling water (3 × 15 min washes). The blot was then hybridized with β2-microglobulin cDNA isolated from Raji cells [13]. The autoradiograms were scanned and the relative amounts of RNA were calculated by normalizing the areas under the RNA peaks against those of β2-microglobulin. Raji-HN2 (lane 1) and Raji (lane 2) poly(A) RNA hybridized with SP-17 (top panel) or β2-microglobulin (β2m; bottom panel). Raji RNA, lacking poly(A), hybridized with SP-17 (lane 3; ribosomal 28*S* RNA = 4.9 kb, 18*S* RNA = 2.0 kb). Raji poly(A) RNA hybridized with SP-12 (lane 4). The positions and sizes (in kb) of RNA markers are shown on the left.

Because treatment of cells with DNA-reactive agents can cause gene alterations [14] (such as rearrangement or amplification), we determined whether such events were induced. The results presented in Fig. 3 show that the cDNAs hybridized with equal efficiency to the same fragments of Raji and Raji-HN2 DNA that were digested with different restriction enzymes. Thus, the increased transcription of topoisomerase II in Raji-HN2 cells were not due to either gene rearrangement or amplification. The chromatin of Raji-HN2 cells is hypersensitive to digestion with deoxyribonuclease I [4]. Hypersensitivity to nuclease digestion is an indication of the presence of active genes and altered chromatin [15]. Consequently, we suggest that the increased transcription of topoisomerase II gene in

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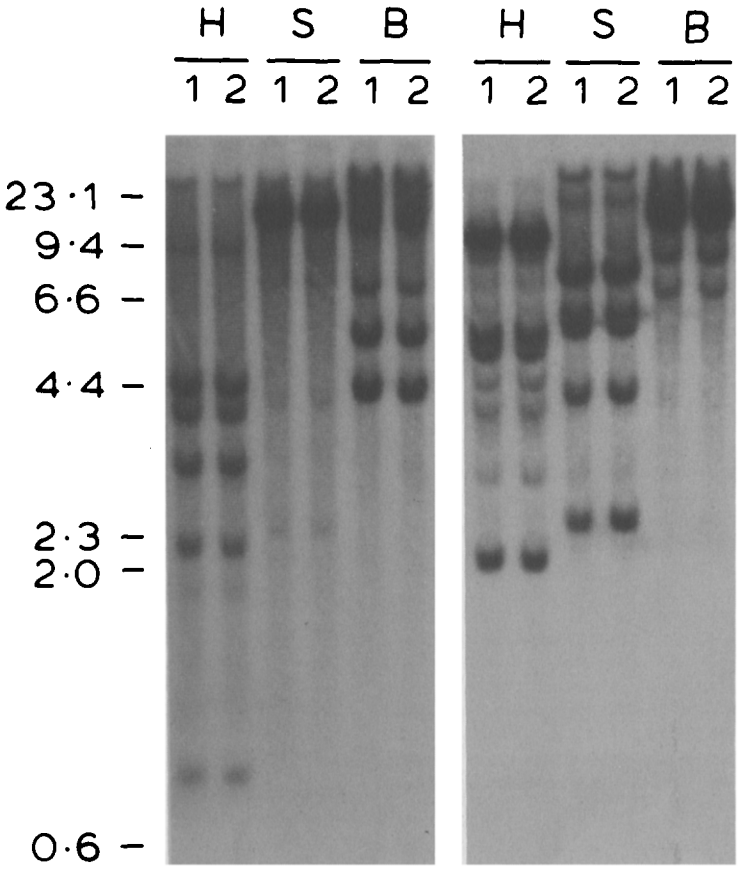

Fig. 3. Blot analysis of Raji-HN2 and Raji DNA. Seven micrograms of Raji-HN2 (1) or Raji (2) DNA digested with Hind III (H), Sst I (S) or Bam HI (B) was fractionated in an agarose gel, blotted to a Zetaprobe membrane, and hybridized to SP-12 (left panel) or SP-17 (right panel) as described in Fig. 2. The positions and sizes (in kb) of λ-Hind III DNA markers are shown on the left.

Raji-HN2 cells resulted from gene activation brought about by changes in chromatin structure.

In summary, Raji-HN2 cells (a Burkitt lymphoma line made resistant to nitrogen mustard) have 3-fold more extractable topoisomerase II activity than the parental Raji cells. Using topoisomerase II antiserum and cDNAs as probes, we found that in Raji-HN2 cells (i) the increased topoisomerase II activity reflected increased enzyme amounts, (ii) transcription of the topoisomerase II gene was increased 2- to 3-fold, and (iii) the topoisomerase II gene was neither amplified nor rearranged.

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