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TOPOISOMERASE-II ACTIVITY IN HUMAN LEUKEMIC AND LYMPHOBLASTOID CELLS

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Topoisomerase-II activity was analyzed in various human leukemic and lymphoblastoid cell-lines with comparison to normal human peripheral blood lymphocytes. All of the examined tumor cells contained this enzyme in both the nuclear and cytoplasmic fractions, whereas no appreciable activity of the enzyme was detected in either fraction of the resting normal lymphocytes. Using pBR322 plasmid as a substrate, undialyzed extracts of the tumor cells exhibited the typical ATP-dependent relaxation of supercoiled circles and formation of linear and catenated structures, as well as the ATP-independent knotting activity. On the other hand, dialyzed extracts exerted only the ATP-dependent supercoil relaxation. Novobiocin inhibited the linearization and catenation but not the supercoil relaxing or knotting activities. This study provides indications for an excessive level of a structurally abnormal topoisomerase-II in these tumor cell-lines.

Accumulating data suggest that, by influencing the topological state of the DNA, topoisomerases I and II may control various biological processes, such as DNA replication (1,2) and recombination (1,3), DNA repair (1,4), chromosome condensation and decondensation(5,6), nucleosome assembly (7), DNA transposition (8) and RNA transcription (1,9, 10). Because of their involvement in the control of so many fundamental biological activities, it is tempting to speculate that these enzymes play an important role in neoplastic cell transformation. The recent observation that certain antitumor drugs act by poisoning topoisomerases (11) seems to support such a view. Therefore, in order to further elucidate this possibility we began a comparative study of these enzymes in tumor and normal cells.

In this paper we present our observations regarding the activity of topoisomerase-II in various human leukemic and lymphoblastoid cell-

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lines. Using the plasmid pBR322 as a substrate, we focused on the following enzymatic reactions: a) ATP-dependent relaxation of the supercoiled circles (1,12); b) ATP-dependent formation of intermediate linear derivative (1); c) ATP-dependent catenation (1); d) ATP-independent knotting activity (1).

MATERIALS AND METHODS

Cells: In this study we used the following human myeloid leukemia cell lines; HL-60 - a premyelocytic leukemia (13), K562 - early blasts and/or erythroblasts (13) and U937 - a monocytic like leukemia (13). Likewise, we used the Daudi and HD-MAR lymphoblastoid B-cell-lines (14). As a normal control we used human peripheral blood lymphocytes. The cells were grown in suspension in RPMI-1640 medium (Gibco), containing 10% fetal calf serum at 37°C in a humidified incubator with 8% CO_2.

Preparation of cytoplasmic and nuclear extracts: This was done as described by Auer et al (15), except that 1mM phenylmethylsulfonyl fluoride (PMSF - a protease inhibitor) and 1mM dithiothreitol (DTT) were added to each of the extracts. When indicated, the cytoplasmic and nuclear extracts were dialyzed against buffers A and B (15) respectively, to which 1mM PMSF and 1mM DTT were added.

Purification of pBR322 plasmid: This plasmid was propagated in E. coli DR-100 and purified as described by Maniatis et al (16) and was used as a substrate for the topoisomerase-II analysis.

Topoisomerase-II assay: Topoisomerse-II was assayed by following the ATP-dependent relaxation, linearization and catenation and the ATP-independent knotting of the supercoiled pBR322 plasmid. The reaction mixture contained in a final volume of 25 µl; 20mM Tris HCl (pH-8.1), 10mM $MgCl_2$, 0.5mM Na_3EDTA, 1mM DTT, 20mM KCl, 10mM Spermidine-Cl-3, 30µg/ml bovine serum albumine, 15% glycerol and 1 µg/ml pBR322 DNA. When indicated, ATP was added at 10mM and novobiocin at 200µg/ml. The reaction was started by adding the cytoplasmic or nuclear extracts at a final protein concentation of 4 µg/ml and terminated after 60 min incubation at 33°C by adding 5µl of stopping buffer giving a final concentration of 5% SDS, 20%glycerol, 0.05% bromophenol blue and 50mM EDTA (pH-8.0). The reaction products were analyzed by gel electrophoresis in 1% agarose horizontal slab gel containing 1 µg/ml ethidium bromide, using a running buffer containing 0.089M Tris HCl, 0.089M boric acid, 0.062M EDTA (pH-8.0) and an electric gradient of 1 volt/cm. The gel was photographed using a short wavelength ultraviolet lamp.

RESULTS

Topoisomerase-II activities in subcellular fractions: In the first experiment we examined the activity of topoisomerase-II in the cytoplasmic and nuclear fractions of the leukemic HL-60 cell-line, compared to that of peripheral blood normal lymphocytes. As can be seen from fig. 1, no appreciable activity was detected in either fraction of the normal lymphocytes (compare lanes 6-9 to lane 1). On the other

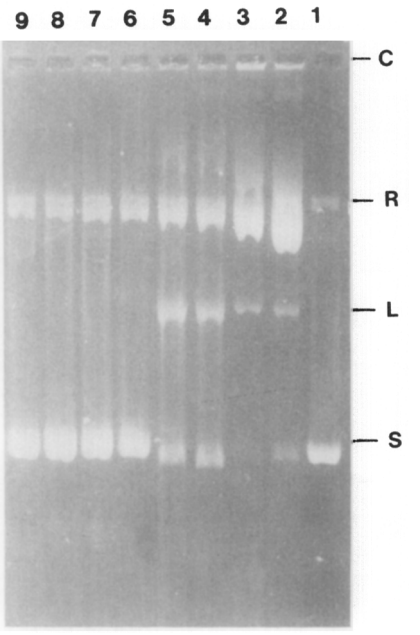

Fig. 1: Comparison of topoisomerase-II-activity in subcellular fractions of HL-60 cells and normal lymphocytes. Topoisomerase-II activity was assay in HL-60 cytoplasmic fraction in the presence (lane 2) and absence (lane 3) of ATP, HL-60 nuclear fraction in the presence (lane 4) and absence (lane 5) of ATP, normal lymphocyte cytoplasmic fraction in the presence (lane 6) and absence (lane 7) of ATP and normal lymphocyte nuclear fraction in the presence (lane 8) and absence (lane 9) of ATP, using supercoiled pBR322 plasmid (shown in lane 1) as substrate. The plasmid structures found at the end of the assay reaction were S = supercoiled; L = linear; R = relaxed and C = catenated.

hand, both the cytoplasmic (lanes 2 and 3) and the nuclear (lanes 4 and 5) fractions of HL-60 cells exhibited a relaxation activity, which proceeded through the formation of a linear intermediate structure. In addition, the HL-60 cytoplasmic, but not the nuclear fraction, showed also a significant catenation activity (top of lanes 2 and 3). Both of these activities are typical for topoisomerase-II. However, they are known to depend on ATP (1), whereas we failed to clearly demonstrate such ATP-dependency with these subcellular fractions (compare lane 2 with 3 and 4 with 5).

Effect of dialysis of the extracts on topoisomerase-II activity:

To elucidate the significance of this apparent ATP-independency, the HL-60 extracts were dialyzed and re-examined. Fig. 2 shows that this

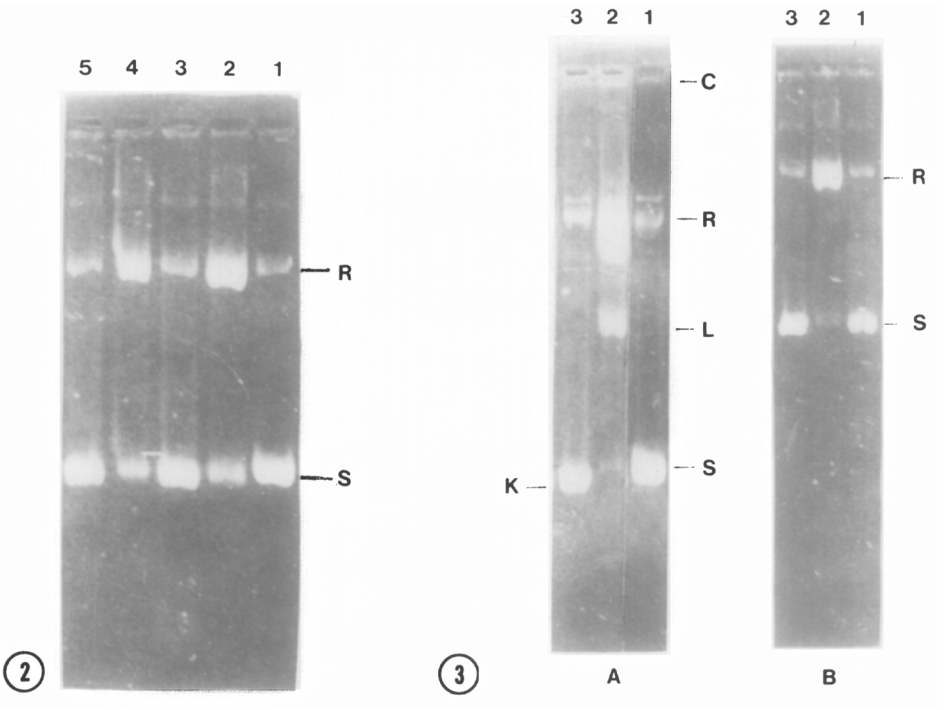

Fig. 2: Effect of dialysis on topoisomerase-II activity of the HL-60 subcellular fractions. Subcellular fractions of HL-60 cells were dialyzed and assayed for topoisomerase-II as follows: Cytoplasmic fraction in the presence (lane 2) and absence (lane 3) of ATP and nuclear fraction in the presence (lane 4) and absence (lane 5) of ATP. The supercoiled pBR322 is shown in lane 1. The plasmid structures are marked as in Fig. 1.

Fig. 3: Topoisomerase-II activity in undialyzed and dialyzed cytoplasmic fraction of U937 lymphoblastoid B-cells. Undialyzed (A) and dialyzed (B) cytoplasmic extracts of U937 cells were assayed for topoisomerase-II activity in the presence (lane 2) and absence (lane 3) of ATP. Lane 1 shows the supercoiled pBR322. The plasmid structures found at the end of the assay reaction were K = knotted; S = supercoiled; L = linear; R = relaxed and C = catenated.

dialysis rendered the enzymatic activity of both extracts dependent on exogenous ATP (compare lane 2 with 3 and 4 with 5), suggesting that before dialysis these extracts contained sufficiently high level of intrinsic ATP. However, with these dialyzed extracts we could no longer detect the intermediate linear DNA, nor the catenation activity (compare lane 2 of fig. 2 with lanes 2 and 3 of fig. 1).

For a further substantiating of these findings we analyzed the topoisomerase-II activity in dialyzed and undialyzed cytoplasmic extracts of the other leukemic and lymphoblastoid cell-lines. Fig. 3 presents the results obtained with U937 cells, but the same results were obta-

ined with all the others. These extracts, in contrast to those of the HL-60 cells, did exhibit a clear ATP-dependency even without being dialyzed (compare lane 2 with 3 of fig. 3A). Furthermore, these cells differ from the HL-60 also in that their undialyzed extracts showed an ATP-independent knotting activity, producing a plasmid derivative with a faster electrophoretic migration than that of the original supercoiled circles (compare lanes1 and 3 of fig 3A). However, after dialysis, these extracts lost all of their activities except the ATP-dependent supercoil relaxation (fig. 3B).

Effect of novobiocin: Novobiocin is a specific inhibitor of topoisomerase-II (17-19). We examined its effect on the activity of this enzyme in the dialyzed and undialyzed cytoplasmic extracts of the various tumor cell-lines. Fig. 4 presents the data obtained with U937

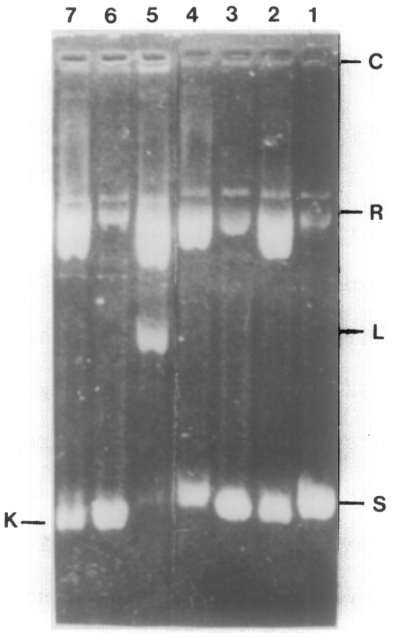

Fig. 4: Effect of novobiocin on topoisomerase-II activity in dialyzed and undialyzed cytoplasmic fraction of U937 cells. Topoisomerase-II was assayed in dialyzed cytoplasmic extracts of U937 cells in the presence (lane 2) and absence (lane 3) of ATP and in the presence of both ATP and novobiocin (lane 4) and in undialyzed cytoplasmic extract of these cells in the presence (lane 5) and absence (lane 6) of ATP and in the presence of both ATP and novobiocin (lane 7). The supercoiled pBR322 is shown in lane 1, and plasmid structures are marked as in Fig. 3.

cell, showing that this drug inhibits the ATP-dependent linearization and catenation by the undialyzed extract (compare lane 5 with 7). However, neither the ATP-dependent relaxation activity of both extracts (compare lane 2 with 4 and 5 with 7) nor the ATP-independent knotting activity of the undialyzed extract (compare lane 6 with 7) were affected. Similar results were obtained with the cytoplasmic extracts of all the other tumor cell-lines.

DISCUSSION

Topoisomerase-II,originally discovered in bacteria (1), has been found also in diverse eukaryotic systems, primarily in replicating cells (1,20-22). Recent studies with regenerating liver (20) and Con A stimulated lymphocytes (21) have shown that the level of topoisomerase-II activity parallels precisely the rate of the cellular DNA replication and the level of DNA polymerase activity. These data strongly suggest a strict cell-cycle dependent control that apparently acts to adjust the topoisomerase-II level to the needs of its function in the nucleus. Indeed in various reported cases, the enzyme has been isolated mainly from the nucleus (19,21,23). In contrast, we found that in additon to the nuclear enzyme, the various tumor cell-lines,tested in our experiments,also contained a remarkable activity of this enzyme in their cytoplasm. This cytoplasmic activity may reflect an abnormal excess of the enzyme resulting, apparently, from an impaired control of its level in such rapidly replicating tumor cells. No appreciable activity of this enzyme was found in either fraction of the normal peripheral blood lymphocytes. This is compatible with other reports showing very low levels of topoisomerase-II in non-activated lymphocytes (21) and other resting cells (20).

The nuclear enzyme of our tumor cell-lines could catalyze only the formation of the intermediate linear structure of the plasmid and its subsequent relaxation, whereas their cytoplasmic enzyme exhibited also catenation and knotting activities. It is unclear whether this

variation reflects two different forms of the enzyme, or an influence of some other factor(s) possibly present in the extracts. Our findings that dialysis remarkably affects the activity pattern of both the nuclear and cytoplasmic enzyme preperatins, seems to rather support the latter possibility.

Dialysis experiments also revealed that the HL-60 extracts contained exceptionally high levels of intrinsic ATP, rendering their topoisomerase-II activities independent on exogenous ATP, and perhaps interfering with its knotting activity, which can be better demonstrated in the absence of ATP.

Finally, we found that novobiocin could inhibit only the linearization and catenation but not the relaxation nor the knotting activities of our extracts. This partial effect of novobiocin contrasts its inhibitory effect on both relaxation and catenation activities found with topoisomerase-II of other eukaryotic cells (17-19). It can therefore be concluded that in addition to its apparently impaired control in the leukemic and lymphoblastoid cells, the enzyme of these cells also carries some abnormal structural modifications.

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