Figure 8

Immunoblotting of DNA topoisomerase IIα and γ-tubulin

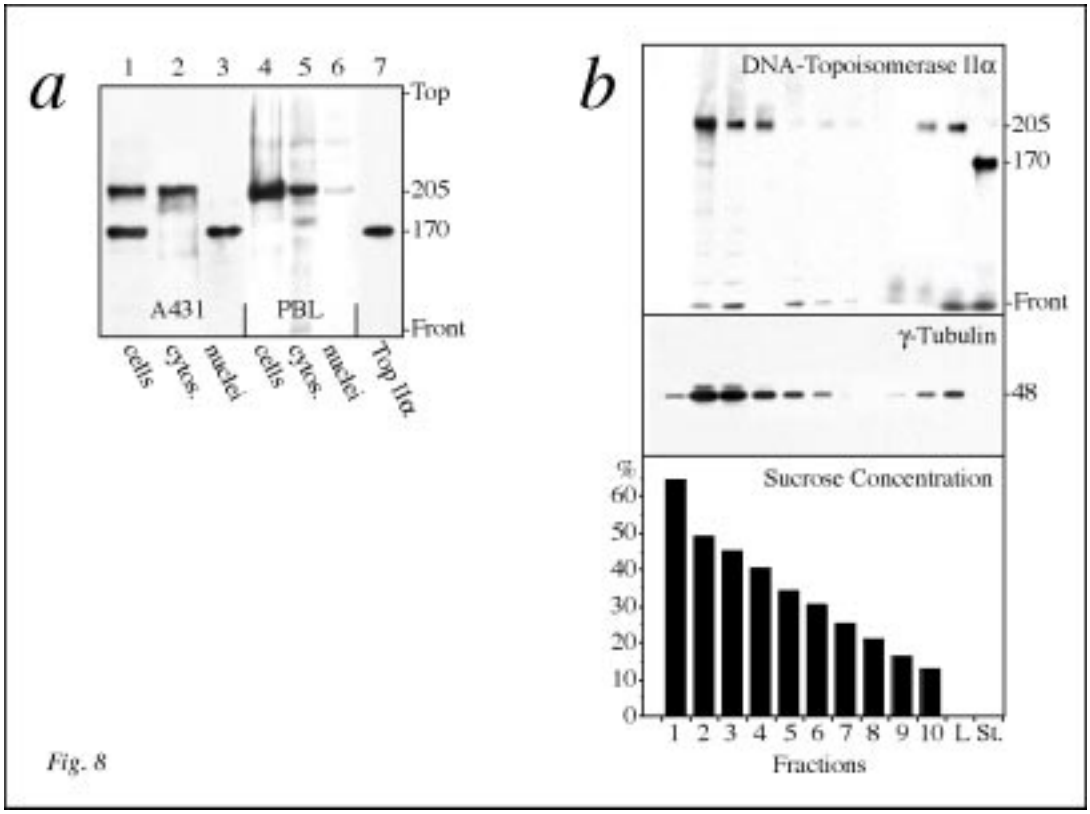

Fig. 8

Figure 8

a: Immunoblotting using rabbit peptide antibodies against the C-terminus of DNA human topoisomerase IIα of whole cell lysate (lanes 1 and 4) or in cytosolic fraction (lanes 2 and 5) or in isolated nuclei (lanes 3 and 6) of A431 (lanes 1-3) and peripheral human blood lymphocytes (lanes 4-6). Each lane contains the equivalent of 10^5 cells and the standard of purified recombinant human DNA-topoisomerase IIα produced in yeast is shown in lane 7.

b: Isolation of centrosomes by isopyknic sucrose gradient density centrifugation. Cytosolic fraction of whole cell lysate A431 cells were sedimented through a sucrose gradient (10-70%,w/v). The gradient was fractionated from the bottom, and these obtained fractions (lanes 1-10) were analysed by immunoblotting using topoisomerase IIα antibody as described above (top) or a rabbit peptide γ-tubulin antibody (middle). The corrosponding sucrose concentration (%,w/v) of the fractions (bottom) was determined by optical diffraction. The two outmost lanes on the right show an aliquot of the cytosolic fraction (L) and of the standard of purified recombinant human DNA-topoisomerase IIα (Std). The Mr-values (kDa) on the right margin were obtained by comparing with marker proteins.