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TOPOISOMERASES: NOVEL THERAPEUTIC TARGETS IN CANCER CHEMOTHERAPY

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DNA topoisomerase II has recently been identified to be an intracellular target of a number of clinically important antitumor drugs such as anthracyclines (e.g. adriamycin), acridines (e.g. m-AMSA), anthracenediones (e.g. mitoxantrone), actinomycins (e.g. actinomycin D), ellipticines (e.g. 2-methyl-9-hydroxyellipticinium acetate) and epipodophyllotoxins (e.g. VP-16 and VM-26)(1-4). Studies both in a purified system using DNA topoisomerase II and in cultured mammalian cells have shown that these antitumor drugs interfere with the breakage-reunion reaction of DNA topoisomerase II by trapping an aborted enzyme-DNA intermediate, termed the "cleavable complex" (1-4). Exposure of the cleavable complexes to strong proteindenaturants such as SDS and alkali results in DNA breakage (both single- and double-strand DNA breaks) and the covalent linking of a topoisomerase II polypeptide to the 5' phosphoryl end of the broken DNA via a tyrosyl phosphate bond (1-3). A brief heating of the drug reaction at 65° C prior to protein denaturant treatment reverses the cleavage reaction both in the purified system and in cultured mammalian cells (5). The mechanism by which these drugs interfere with the topoisomerase II reaction is shown in Fig. 1.

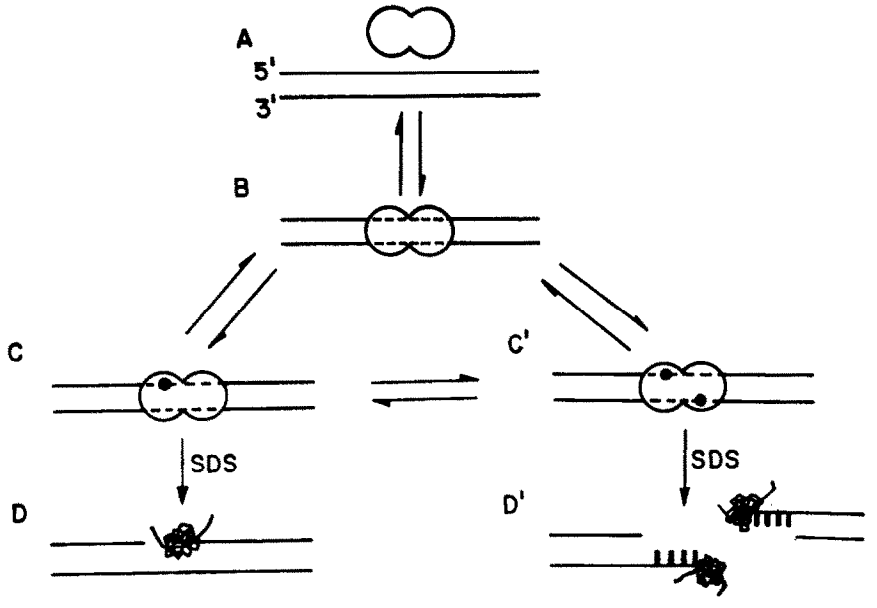

Fig. 1. Proposed mechanism of action of topoisomerase II-specific antitumor drugs. B, non-cleavable complex. C and C', cleavable complex. DNA topoisomerase II binds DNA by forming at least two types complexes, the non-cleavable complex (B) and the cleavable complex (C and C'), which are at equilibrium. The equilibrium concentrations of cleavable complexes (C and C') are very low relative to the concentration of non-cleavable complex (B). Topoisomerase II-specific antitumor drugs presumably interact with the enzyme-DNA complexes and alter the equilibrium by increasing the concentration of the cleavable complexes (C and C'). Exposure of the cleavable complexes to strong protein denaturants results in single- and double-strand DNA breaks and the covalent linking of a topoisomerase II polypeptide (Mr=170 Kd) to each 5' phosphoryl end of the broken DNA strand.

The possibility that topoisomerase II-mediated DNA breaks may be responsible for cytotoxicity has been investigated using a number of m-AMSA-related acridines. The levels of topoisomerase II-

mediated DNA breaks induced by these acridine compounds in the purified system strongly correlate with the levels of protein-linked DNA breaks in cultured cells (6). Furthermore, the levels of protein-linked DNA breaks, which reflect the amounts of the reversible cleavable complexes, also strongly correlate with the cytotoxicity and antitumor activity of these acridines (6). While all evidence thus far indicate that drug-trapped cleavable complexes are primarily responsible for the drug action during an acute exposure, the reversibility of the cleavable complexes suggests that other cellular processes must interact with the cleavable complexes to signal cell death and other cellular responses (7).

The level of DNA topoisomerase II is predictably one of the important factor in determining drug cytotoxicity. Studies in a number of normal and neoplastic cells have shown that the level of DNA topoisomerase II is regulated by both serum growth factors and the cell density (8). While the level of DNA topoisomerase II is tightly regulated by serum growth factors (shown in Fig. 2) and the cell density in normal cells (8), it is much less sensitive to these growth conditions in transformed cells (8). Using synchronized HeLa cells, the level of topoisomerase II has been shown to remain relatively constant throughout G1, S, G2 and M phases of the cell cycle (8). These results suggest that the level of DNA topoisomerase II is primarily regulated during the entry of quiescent cells into the proliferative state.

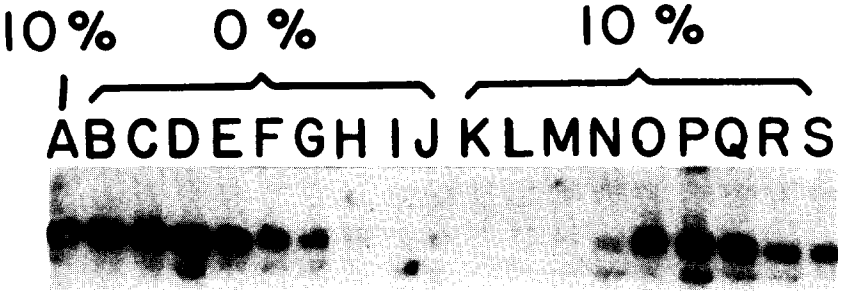

Fig. 2. Effect of serum on the level of DNA topoisomerase II in cultured mouse 3T3 cells. NIH 3T3 cells were grown in Eagle's minimum essential medium (MEM) supplemented with 10% bovine serum. After reaching confluency, cells were serum-starved in serum-free MEM for up to 72 hrs and then stimulated to grow in 10% serum. Cells were sampled every 8 hours and whole cell lysates (about 10^6 cells/lane) were analyzed for topoisomerase II content by immunoblotting with topoisomerase II-specific antisera.

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