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Multilevel Therapeutic Targeting by Topoisomerase Inhibitors

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Summary The successful use of cytotoxic agents in the clinical management of LCH depends upon the selective targeting of cells participating in the disease process. The topoisomerase 'poisons', currently used extensively in the treatment of aggressive malignancies, represent an intriguing class of cytotoxic agents exerting their cytostatic and cytotoxic effects at multiple levels according to cell type. The non-DNA intercalating topoisomerase II poison, etoposide (VP-16), is the "drug of first choice" in the treatment of LCH by cytotoxic chemotherapy. This major anticancer agent traps the nuclear enzyme DNA topoisomerase II on DNA in a sequence-specific manner, the processing of trapped complexes giving rise to a plethora of cellular effects not least the potential activation of pathways leading to cell cycle arrest and apoptosis. This short review describes the principles of topoisomerase inhibition, the multiplicity of cellular effects and the concept of cellular targeting in LCH. The successful treatment of LCH by cytotoxic chemotherapy will depend on both the identity of the target tissues and a clear view of therapeutic intent, given the potential for induction of haematological neoplasia.

Introduction

The eukaryotic cell has the formidable problem of maintaining control over the higher order structure of its genetic material, while pursuing the biological imperatives of DNA replication, transcription and the separation of daughter chromosomes at cell division. The orderly progression of these cellular processes is dependent upon the action of the nuclear topoisomerases – enzymes that resolve topological and conformational changes in DNA (Wang, 1985; Liu, 1989). Inhibition of topoisomerases can disrupt cell cycle progression and generate intracellular signals that lead to cell death or prolonged cytostasis (Smith, 1990) so it is not surprising to find that several classes of anticancer drugs are now recognised as potent topoisomerase "poisons". The epipodophyllotoxins are the most widely used class of non-intercalative antitumour topoisomerase II poisons. Etoposide (VP-16), in particular, is currently used in the treatment of a number of adult and child cancers eg. acute leukaemia, non-Hodgkin lymphomas and Hodgkin disease, malignant germ cell tumours, small cell lung cancer and neuroblastoma. The effectiveness of topoisomerase inhibitors in modifying the *in vivo* behaviour and the viability of targeted cell populations is relevant to the clinical management of LCH because etoposide has also become a 'drug of first choice' in the management of the 2 principle forms of histiocytosis – Langerhans cell histiocytosis (LCH) and haemophagocytic lymphohistiocytosis. To place the rational application of chemotherapy in LCH into context, this selective review considers the principles of topoisomerase inhibition and some of the factors governing cellular responses to inhibition.

Topoisomerases: forms, functions and regulation

Through highly co-ordinated processes topoisomerases permit changes in the topological state of compromised DNA molecules as shown diagrammatically in Figure 1. The topoisomerases have been classified as either type I or type II enzymes depending on their ability to cleave one or two DNA strands, respectively.

The human type I enzyme, topoisomerase I, can relax supercoiled DNA by a process involving the transient introduction of a single-strand break in DNA and the passing of an intact strand through the gap prior to re-ligation. This reaction is independent of ATP supply. Topoisomerase II can unknot, decatenate or relax supercoiled DNA by the transient introduction of a double-strand break through which an intact helix can be passed, the complete reaction requiring ATP hydrolysis (Wang, 1985; Liu, 1989).

The human topoisomerase I enzyme, a monomer of approximately 95 kDa, is encoded by a single copy gene located on human chromosome 20 (Juan *et al.*, 1988). The human type II enzyme, DNA topoisomerase II, is found in two forms encoded by single copy genes yielding homodimeric proteins of 170 kDa (topoisomerase 11α) and 180 kDa (topoisomerase IIβ) (Tan *et al.*, 1992). The human chromosomal location of the topoisomerase IIα gene is at 17q21-22 (Tsai-Pflugfelder *et al.*, 1988) and the topoisomerase II gene has been mapped to 3p24 (Jenkins *et al.*, 1992). While the expression of the topoisomerases I and IIβ is the same in quiescent and cycling cells, the expression of the topoisomerase IIαk is low in G0-G1 cells but high in proliferating and transformed cells (Heck *et al.*, 1988, Woessner *et al.*, 1990). In contrast, topoisomerase IIβ is preferentially expressed in quiescent cells (Woessner *et al.*, 1990). Topoisomerase IIα shows a cycle of synthesis and degradation coupled to, or perhaps driving, changes in chromosome condensation and decondensation (Heck *et al.*, 1988). Both DNA topoisomerases I and II undergo post-translational modification and are poly (ADP)-ribosylated, serine-phosphorylated proteins (Kroll & Rowe, 1991; Jenkins *et al.*, 1992). The phosphorylation of the topoisomerase II increases activity and its phosphorylation status follows its cell cycle-dependent expression (Heck *et al.*, 1988; Saijo *et al.*, 1990; Saijo *et al.*, 1992).

Topoisomerase I aids the orderly progression of DNA replication, transcription and recombination and shows strong sequence preferences for DNA binding and cleavage. The type I enzyme is involved in the repression and activation of transcription (Merino *et al.*, 1993) and is therefore more abundant in transcriptionally-active regions of the nucleus. It has an essential role in higher eukaryote development (Lee *et al.*, 1993). Unlike the type I enzyme, topoisomerase II is essential for cell viability and facilitates the resolution and separation of daughter chromosomes at mitosis. The more abundant isoform, topoisomerase IIα, is located at nuclear matrix-associated regions, a strategic location at the base of chromatin loop domains which infers both structural and functional roles (Wood & Earnshaw, 1990). Topoisomerase IIβ is found predominantly in the nucleolus (Negri *et al.*, 1992; Zini *et al.*, 1992) and binds to GC-rich sequences present in gene promotor regions, suggesting participation in gene expression (Ura & Hirose, 1991).

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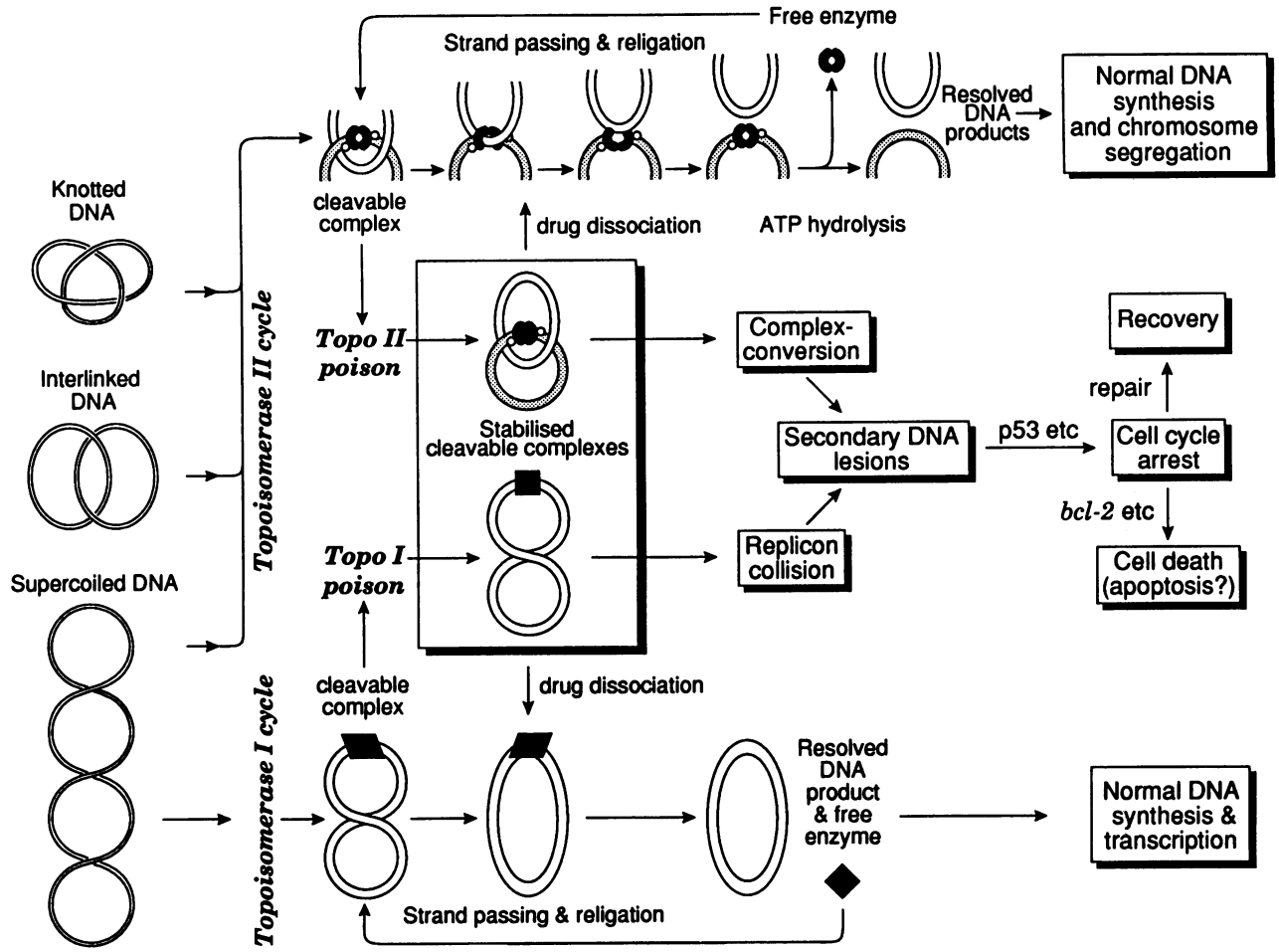

Figure 1 Diagrammatic representation of the normal activities of type I and type II topoisomerases, the effects of specific poisons and the cellular consequences of trapping topoisomerase-DNA complexes

Targeting of topoisomerases by anticancer drugs

Several classes of antitumour drugs can act as topoisomerase poisons and there is now convincing evidence that DNA topoisomerases are important, and perhaps primary, cellular targets (Liu 1989; Schneider *et al.*, 1990). The Figure shows how poisons stabilise the topoisomerase-DNA complex in a form that can be cleaved by strong protein denaturants to reveal that, within the complex, the trapped enzyme sequesters a DNA single- or double-strand break. Not all topoisomerase inhibitors act to trap cleavable complexes (Cummings & Smyth, 1993). For example, the disruption of ATP turnover by coumarins (eg novobiocin) can inhibit the activity of the type II enzyme. Derivatives of the antitumour plant alkaloid camptothecin have been found to be specific topoisomerase I poisons that trap complexes (Ryan *et al.*, 1991; Madden & Champoux, 1992; Slichenmyer *et al.*, 1993). Topoisomerase II poisons include both DNA-intercalating and non-intercalating agents: isoflavones (eg. genistein), quinolones (eg. CP-115,953), ellipticines (eg. 9-hydroxyellipticinium), anthracyclines (eg. doxorubicin), anilinoacridines (eg. amsacrine) and epipodophyllotoxins (eg. etoposide and teniposide). The current view is that the topoisomerase II molecule has domains for interaction with poisons and that the interaction domain of etoposide overlaps those of several cleavage-enhancing drugs (Corbett *et al.*, 1993).

Targeting of DNA damage by topoisomerase poisons

Topoisomerase poisons can target complex-trapping on DNA in a manner dependent upon sequence, gene activity, nuclear location and function. Strong topoisomerase II binding sites on DNA have been described (Spitzner *et al.*, 1990; Huang *et al.*, 1992) with flanking recognition sequences (Fosse *et al.*, 1988). Topoisomerase poisons in the same chemical class tend to trigger the cleavage of DNA at similar sites (Pommier *et al.*, 1991). In general the overall capacity to trap complexes in intact cells is correlated with the cytotoxicity of a poison within a given chemical class (Long *et al.*, 1984).

The stabilisation by antitumour drugs of cleavable complexes in the promotor regions of genes and interference with transcription complexes could explain the decreased expression of specific genes observed in drug-treated cells. For example, teniposide and etoposide induce damage preferentially around and within the c-myc proto-oncogene rather than randomly in the whole genome (Riou *et al.*, 1989; Gewirtz *et al.*, 1993) and, surprisingly, transcriptionally inactive β-globin genes are more sensitive to epipodophyllotoxin-induced damage than bulk DNA (Gewirtz *et al.*, 1993). The accessibility and conformation of DNA within the nucleus may also dictate the intranuclear location of topoisomerase trapping by poisons. Teniposide and amsacrine appear to interact preferentially with nuclear matrix-bound topoisomerase II, and one mechanism of cellular resistance to these agents may relate to modified intracellular localisation (Feldhoff *et al.*, 1994). Studies of the interactions of anticancer agents with targets in the nuclear matrix should provide further insight into the mechanisms by which these agents exert their therapeutic effects (Fernandes & Catapano, 1991). Thus, cell type-dependent antitumour activity of a topoisomerase poison may relate to damage to specific genes and/or to features of nuclear architecture specific to that cell type.

Cell targeting by topoisomerase poisons

The selectivity of anticancer agents is often related to the increased proliferation rate of tumour cells. Although co-ordinated topoisomerase activity may be vital to proliferating cells, the cytotoxic action of topoisomerase poisons is associated with the presence of trapped complexes rather than the withdrawal of enzyme function per se. The intrinsic sensitivity of actively proliferating cells to a topoisomerase poison is crucially dependent upon the availability of the target enzyme (Liu 1989; Smith & Makinson, 1989; Webb *et al.*, 1991) as determined by cell cycle disposition and topoisomerase gene expression. Sensitivity to the topoisomerase I poison camptothecin is dependent upon the amount of enzyme and active DNA replication. The requirement for DNA replication is consistent with a replicon-collision model for the generation of double-strand breaks. These 'secondary' DNA lesions lead to inhibition of DNA synthesis, arrest of cells in G2 and cell death (Hsiang *et al.*, 1989; Falk & Smith, 1992). In contrast, RNA and protein synthesis have been shown to be necessary to the expression of etoposide- (Chow *et al.*, 1988) and amsacrine-induced (Schneider *et al.*, 1989) cytotoxicity, suggesting that the processing or conversion of trapped topoisomerase II complexes may also give rise to secondary DNA lesions perhaps through an activation of endonucleolytic degradation (Kaufmann, 1989).

In human colon cancer, a tumour type particularly sensitive to camptothecin, there is an elevated level of type I enzyme (Giovanella *et al.*, 1989). Interestingly, tumour cells, even those of the same histogenetic orgin, can vary in the degree of expression of topoisomerase II. For example, ten-fold variation was found in a recent study of human lung cancer cell lines (Giaccone *et al.*, 1992). Furthermore, there may be differential expression of the type I and II enzymes in tumour and normal tissues, implying that selectivity could be achievable for one class of poisons. Indeed, it has been reported that while lung cancer and normal lung tissues show identical topoisomerase I mRNA patterns there is enhanced expression of topoisomerase II mRNA in the tumour cells (Hasegawa *et al.*, 1993). As yet there is no clear consensus as to the relevance of the topoisomerase II enzyme for cytotoxicity or whether given tumour types display distinctive patterns of expression (Jenkins et al., 1992).

LCH and etoposide

The potential for low doses of topoisomerase II poisons to produce quasi-synchronisation of target populations, rather than immediate cytostasis and cell death, is a relatively unexplored aspect of 'cell targeting'. Therapy-related dynamic changes in target cell populations may have several consequences. Low-dose therapy may reduce the cell proliferation rate while enhancing the intrinsic chemo-sensitivity of cells by increasing the availability of a cell cycle-regulated target enzyme such as topoisomerase II. Studies of etoposide in the treatment of small cell lung cancer have provided evidence of schedule-dependency (Dombernowsky & Nissen, 1973; Wolfe *et al.*, 1987). Continuous low concentrations of etoposide may be required for optimal activity of the drug when administered as a single agent (Clark *et al.*, 1989). The optimal use of etoposide against target cells in LCH is also likely to be highly schedule-dependent.

The induction of haematological neoplasia is a conspicuous problem with the use of chemotherapeutic agents in the management of human disease. Indeed, acute nonlymphocytic leukaemia (ANLL) is a well-recognised complication of regimens incorporating alkylating agents. The leukaemogenic potential of topoisomerase poisons, particularly the epipodophyllotoxins, has been more difficult to assess because they are usually used in combination chemotherapy rather than as single agents. However, recent evidence (Whitlock *et al.*, 1991; Pederson-Bjergaard *et al.*, 1992) supports a relationship between the use of epipodophyllotoxins and the induction of secondary ANLL with characteristics distinct from that occurring after treatment with alkylating agents. Furthermore, topoisomerase II-interactive anthracyclines may contribute to leukaemogenesis by acting synergistically with directly-acting genotoxic drugs. To date the leukaemogenic potential of low-dose single-agent etoposide therapy has not been determined.

The optimal therapeutic approach in the treatment of LCH is not clear. Should therapy aim to selectively destroy abnormal cells or to modify the behaviour of affected tissues and cellular subpopulations? On the one hand, the use of topoisomerase inhibitors in doses and schedules usually employed to effect tumour cell destruction may run the risk of induction of ANLL. The risk may be reduced by the use of single-agent/prolonged-schedule regimens but confirmation awaits further clinical studies. On the other hand, aberrant cellular processes in LCH may be subject to selective therapeutic modulation by the disturbance of topoisomerase function, although it is likely that such processes would have to impinge upon DNA metabolism in some way.

It is becoming increasingly clear that the response of a tumour cell to topoisomerase inhibition is not dependent upon topoisomerase-targeting alone. Rather, the integrity of cellular 'checkpoints' controlling cell cycle traverse and the function of elements responsive to DNA damage and stalled DNA metabolism play important roles in determining the cytostatic and cytotoxic actions of topoisomerase poisons ((Kastan *et al.*, 1992; Kaufmann, 1989; Lock and Ross, 1990; Dive *et al.*, 1992). Arrest at the G1/S phase and G2/mitosis cell cycle stages are typical but variable responses of proliferating human cells to DNA damaging agents. These cell cycle checkpoints can be viewed as the nodes at which systems capable of detecting cellular stress, in this case DNA damage, interface with the mechanisms controlling cell cycle traverse (Lane, 1993, Lock and Ross, 1990).

Studies have shown a requirement for the product of the *p53* tumour supressor gene both in the efficient activation of apoptosis (Clarke *et al.*, 1993) and in the arrest of cells at the G1/S phase checkpoint (Kastan *et al.*, 1992). The general concept is that *p53* can act as a guardian of the genome (Lane, 1993) and provide a defence reaction against the consequences of uncontrolled replication of DNA carrying lesions induced by genotoxic agents. This defence involves the activation of a block to S phase entry, providing a potential recovery period, and a programmed apoptotic destruction of damaged cells. In the presence of a functional *p53*-dependent pathway, cells appear to be capable of expressing apoptosis with evidence that *c-myc* and *bcl-2* genes cooperate in that process of active cell death (Evan *et al.*, 1992; Fanidi *et al.*, 1992). Critically, checkpoint modulation can alter cellular responsiveness to chemotherapeutic agents (Lowe *et al.*, 1993) and topoisomerase poisons are effective inducers of apoptosis (Bertrand *et al.*, 1993). However, there is currently no evidence that checkpoint abnormalities are a feature of LCH and it would be expected that such cells are competent for both cell cycle arrest and the ability to commit damaged cells to apoptosis, although this awaits experimental confirmation.

Persistent cytostasis induced by topoisomerase poisons, reflecting chronic checkpoint activation, may be a valuable clinical outcome in the control of abnormal cellular behaviour including LCH. The future for cytotoxic chemotherapy of LCH my lie in the selection and application of cytotoxic agents, such as etoposide, to attain a level of selectivity for given cell lineages combined with the complementary manipulation of response pathways that alter the population dynamics of the target cells through the promotion of cell cycle arrest and cell loss.

References

BERTRAND, R., SOLARY, E., JENKINS, J., & POMMIER, Y. (1993). Apoptosis and its modulation in human promyelocytic HL60 cells treated with DNA topoisomerase I and II inhibitors. *Exp. Cell Res.*, **207**, 388–397.

CHOW K.C., KING C.K. & ROSS W.E. (1988) Abrogation of etoposide-mediated cytotoxicity by cycloheximide. *Biochem.Pharmacol.*, **37**, 1117–1122.

CLARKE, A.R., PURDIE, C.A., HARRISON, D.J., MORRIS, R.G., BIRD, C.C., HOOPER, M.L., & WYLLIE, A.H. (1993). Thymocyte apoptosis induced by p53-dependent and independent pathways. *Nature*, **362**, 849–852.

CLARK, P.I., JOEL, S.P. & SLEVIN, M.L. (1989) A pharmacokinetic hypothesis for the clinical efficacy of etoposide in small cell lung cancer. *Proc Am Soc Clin Oncol*, **8**, 66 (Abstract).

CORBETT, A.H., HONG, D. & OSHEROFF, N. (1993) Exploiting mechanistic differences between drug classes to define functional drug interaction domains on topoisomerase II. *J. Biol. Chem.*, **268**, 14394–14398.

CUMMINGS, J. & SMYTH, J.F. (1993) Topoisomerase I and II as targets in cancer chemotherapy. *Cancer Topics*, **9**, 75-77.

DIVE, C., EVANS, C.A. & WHETTON, A.D. (1992) Induction of apoptosis -new targets for cancer chemotherapy. *Seminars in Cancer Biology*, **3**, 417–427.

DOMBERNOWSKY, P. & NISSEN, N.I. (1973) Schedule dependency of the antileukaemic activity of the podophyllotoxin-derivative VP16-213 (NSC-141540) in L1210 leukaemia. *Acta Path. Microbiol. Scand.*, **81**, 715–724.

EVAN, G.I., WYLLIE, A.H., GILBERT, C.S., LITTLEWOOD, T.D., LAND, H., BROOKS, M., WATERS, C.M., PENN, L.Z., & HANCOCK, D.C. (1992). Induction of apoptosis in fibroblasts by c-myc protein. *Cell*, **69**, 119–128.

FANIDI, A., HARRINGTON, E.A., & EVAN, G.I. (1992). Cooperative interaction between c-myc and bcl-2 proto-oncogenes. *Nature*, **359**, 554–556.

FALK, S.J. & SMITH, P.J. (1992) DNA damaging and cell cycle effects of the topoisomerase I poison camptothecin in irradiated human cells. *Int. J. Radiat. Biol.*, **61**, 749–757.

FERNANDES, D.J. & CATAPANO, C.V. (1991). Nuclear matrix targets for anticancer agents. *J. Cancer Cells*, **3**, 134–140.

FOSSE, P., PAOLETTI, C., & SAUCIER, J.M. (1988) Pattern of recognition of DNA by mammalian DNA topoisomerase II. *Biochem. Biophys. Res. Commun.*, **151**, 1233–1240.

GEWIRTZ, D.A., ORR, M.S., FORNARI, F.A., RANDOLPH, J.K., YALOWICH, J.C., RITKE, M.K., POVIRK, L.F. & BUNCH, R.T. (1993) Dissociation between bulk damage to DNA and the antiproliferative activity of teniposide (VM-26) in the MCF-7 breast tumor cell line: evidence for induction of gene-specific damage and alterations in gene expression. *Cancer Res.*, **53**, 3547–3554.

GIACCONE, G., GAZDAR, A.F., BECK, H., ZUNINO, F. & CAPRANICO G. (1992) Multidrug sensitivity phenotype of human lung cancer cells associated with topoisomerase II expression. *Cancer Res.*, **52**, 1666–1674.

GIOVANELLA, B.C., STEHLIN JR. J.S., WALL, M.E., WANI M.C., NICHOLAS, A.W., LIU L.F., SILBER R. & POTMESIL M. (1989) DNA topoisomerase I targeted chemotherapy of human colon cancer in xenografts. *Science*, **246**, 1046–1048.

HASEGAWA, T., ISOBE, K-I., NAKASHIMA, I. & SHIMOKATA, K. (1993) Higher expression of topoisomerase II in lung cancers than normal lung tissues: different expression pattern from topoisomerase I. *Biochem. Biophys. Res. Commun.*, **195**, 409–414.

HECK, M.M., HITTELMAN, W.N. & EARNSHAW, W.C. (1988) Differential expression of DNA topoisomerases I and II during the eukaryotic cell cycle. *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 1086–1090.

HSIANG, Y.H., LIHOU, M.G. & LIU L.F. (1989) Arrest of replication forks by drug stabilized topoisomerase I-DNA cleavage complexes as a mechanism of cell kiling by camptothecin. *Cancer Res.*, **49**, 3230–3235.

HUANG, H.W., JUANG, J.K. & LIU H.J. (1992) The recognition of DNA cleavage sites by porcine spleen topoisomerase II. *Nucleic Acid Res.*, **20**, 467–473.

JENKINS, J.R., AYTON, P., JONES, T., DAVIES S.L., SIMMONS, D.L., HARRIS, A.L., SHEER, D. & HICKSON, D. (1992) Isolation of cDNA clones encoding the isoenzyme of DNA topoisomerase II and localisation of the gene to chromosome 3p24. *Nucleic Acid Res.*, **20**, 5587–5592.

JUAN, C.C., HWANG, J., LIU, A.A., WHANG-PENG, J., KNUTSEN, T., HUEBNER, K., CROCE, C., ZHANG, H., WANG, J.C., & LIU, L.F. (1988) Human DNA topoisomerase I is encoded by a single copy gene that maps to chromosome region 20q12-13.2. *Proc. Natl. Acad. Sci., USA*, **85**, 8910–8913.

KASTAN, M.B., ZHAN, Q., EL-DEIRY, W.S., CARRIER, F., JACKS, T., WALSH, W.V., PLUNKETT, B.S., VOGELSTEIN, B. & FORNACE, A.J. (1992) A mammalian cell cycle checkpoint pathway utilizing p53 and GADD45 is defective in ataxia-telangiectasia. *Cell*, **71**, 587–597.

KAUFMANN, S. (1989) Induction of endonucleolytic DNA cleavage in human acute myelogenous leukaemia by etoposide, camptothecin, and other anticancer drugs. *Cancer Res.*, **49**, 5870–5878.

KROLL, D.J. & ROWE, T.C. (1991) Phosphorylation of DNA topoisomerase II in a human tumor cell line. *J. Biol. Chem.*, **266**, 7957–7961.

LANE, D.P. (1992) p53, guardian of the genome. *Nature*, **358**, 15–16.

LEE, P.L., BROWN, S.D., CHEN, A. & HSIEH, T.H. (1993) DNA topoisomerase I is essential in Drosophila melanogaster. *Proc. Natl. Acad. Sci. USA*, **90**, 6656–6660.

LIU, L.F. (1989) DNA topoisomerase poisons as anti-tumour drugs. *Ann. Rev. Biochem.*, **58**, 351–377.

LOCK, R.B. & ROSS, W.E. (1990) Possible role for p34cdc2 kinase in etoposide-induced cell death of Chinese hamster ovary cells. *Cancer Res.*, **50**, 3767–3771.

LONG, B.H., MUSIAL, S.T. & BRATTAIN, M.G. (1984) Comparison of cytotoxicity and DNA breakage activity of congeners of podophyllotoxin including VP16-213 and VM26: A quantitative structure-activity relationship. *Biochem.*, **23**, 1183–1188.

LOWE, S.W., RULEY, H.E., JACKS, T., & HOUSMAN, D.E. (1993). p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell*, **74**, 957–967.

MADDEN, K.R. & CHAMPOUX, J.J. (1992) Overexpression of human topoisomerase I in baby kidney cells: hypersensitivity of clonal isolates to camptothecin. *Cancer Res.*, **52**, 525–532.

MERINO, A., MADDEN, K.R., LANE, W.S., CHAMPOUX, J.J. & REINBERG, D. (1993) DNA topoisomerase I is involved in both repression and activation of transcription. *Nature*, **365**, 227–232.

NEGRI, C., CHIESA, R., CERINO, A., BESTAGNO, M., SALA, C., ZINI, N., MARALDI, N.M. & ASTALDI RICOTTI, G.C.B., (1992) Monoclonal antibodies to human DNA topoisomerase I and the two isoforms of DNA topoisomerase II: 170- and 180-kDa isozymes. *Exp. Cell Res.*, **200**, 452-459.

PEDERSON-BJERGAARD, J., SIGSGAARD, T.N., NIELSEN, D., GJEDDE, S.B., PHILIP, P., HANSEN, M., LARSEN, S.O., RRTH, M., MOURIDSEN, H., & DOMBERNOWSKY, P. (1992) Acute monocytic or myelomonocytic leukemia with balanced chromosome translocations to band 11q23 after therapy with 4-epi-doxorubicin and cisplatin of cyclophosphamide for breast cancer. *J. Clin. Oncol.*, **10**, 1444–1451.

POMMIER, Y., CAPRANICO, G., ORR, A. & KOHN, K.W. (1991) Local base sequence preferences for DNA cleavage by mammalian topoisomerase II in the presence of amsacrine and teniposide *Nucleic. Acids Res.*, **19**, 5973–5980.

RIOU, J.F., LEFEVRE, D. & RIOU, G. (1989) Stimulation of the topoisomerase II induced DNA cleavage sites in the c-myc proto-oncogene by antitumor drugs is associated with gene expression. *Biochem.*, **28**, 9104–9110.

RYAN, A.J., SQUIRES, S., STRUTT, H.L. & JONHSON, R.T. (1991) Camptothecin cytotoxicity in mammalian cells is associated with the induction of persistent double strand breacks in replicating DNA. *Nucleic. Acids Res.*, **19**, 3295–3300.

SAIJO, M., ENOMOTO, T., HANAOKA, F. & UI, M. (1990) Purification and characterization of type II DNA topoisomerase from mouse FM3A cells: phosphorylation of DNA topoisomerase II and modification of its activity. *Biochem.*, **29**, 583–590.

SAIJO, M., UI, M. & ENOMOTO, T. (1992) Growth state and cell cycle dependent phosphorylation of DNA topoisomerase II in Swiss 3T3 cells. *Biochem.*, **31**, 359–363.

SCHNEIDER, E., HSIANG, Y.H. & LIU, L.F. (1990) DNA topoisomerases as anticancer drug targets. In: *Advances in Pharmacology*, 21, (August T., Anders M.W., Murad F., & Nies A., eds). Academic Press, Inc., San Diego, Boston, London, Sydney, Tokyo, Toronto, p. 149–183.

SCHNEIDER, E., LAWSON, P.A., & RALPH, R.K. (1989) Inhibition of protein synthesis reduces the cytotoxicity of 4'(9-acridinylamino)methanesulfon- m -aniside without affecting DNA breakage and DNA topoisomerase II in a murine mastocytoma cell line. *Biochem. Pharmacol.*, **38,** 263–269.

SLICHENMYER, W.J. ROWINSKY, E.K., DONEHOWER, R.C . & KAUFMANN, S.H. (1993) The current status of camptothecin analogues as antitumor agents. *JNCI*, **85,** 271–91.

SMITH, P.J. (1990) DNA topoisomerase dysfunction: a new goal for antitumor chemotherapy. *Bio Essays*, **12,** 167–172.

SMITH, P.J. & MAKINSON, T.A. (1989) Cellular consequences of overproduction of DNA topoisomerase II in an ataxia-telangiectasia cell line. *Cancer Res.*, **49,** 1118–1124.

SPITZNER, J.R., CHUNG, I.K. & MULLER M.T. (1990) Eukaryotic topoisomerase II preferentially cleaves alternating purine-pyrimidine repeats. *Nucleic Acid Res.*, **18,** 1–11.

TAN, K.B., DORMAN, T.E., FALLS, K.M., CHUNG, T.D., MIRABELLI, C.K., CROOKE, S.T. & MAO, J. (1992) Topoisomerase IIk and topoisomerase II genes: characterization and mapping to human chromosomes 17 and 3, respectively. *Cancer Res.*, **52,** 231–234.

TSAI-PFLUGFELDER, M., LIU, L.F., LIU, A.A., TEWEY, K.M., WHANG-PENG, J., KNUTSEN, T., HUEBNER, K., CROCE, C.M. & WANG, J.C. (1988) Cloning and sequencing of cDNA encoding human DNA topoisomerase II and localization of the gene to chromosome region 17q21-22. *Proc. Natl. Acad. Sci. USA*, **85,** 7177–7181.

URA, K. & HIROSE, S. (1991) Possible role of DNA topoisomerase II on transcription of the homeobox gene Hox-2.1 in F9 embryonal carcinoma cells. *Nucleic Acid Res.*, **19,** 6087–6092.

WANG, J.C. (1985) DNA topoisomerases. *Annu. Rev. Biochem.*, **54,** 665–697.

WEBB, C.D., LATHAM, M.D., LOCK, R.B. & SULLIVAN, D.M. (1991) Attenuated topoisomerase II content directly correlates with a low level of drug resistance in a Chinese hamster ovary cell line. *Cancer Res.*, **51,** 6542–6549.

WHITLOCK, J.A., GREER, J.P., & LUKENS, J.N. (1991) Epipodophyllotoxin-related leukemia. *Cancer*, **68,** 600–604.

WOESSNER, R.D., CHUNG, T.D.Y., HOFMANN, G.A., MATTERN, M.R., MIRABELLI, C.K., DRAKE, F.H., & JOHNSON, R.K. (1990) Differences between normal and ras- transformed NIH-3T3 cells in expression of the 170 kD and 180 kD forms of topoisomerase II. *Cancer Res.*, **50,** 2901–2908.

WOLFE, S.N., GROSH, W.W., PRATER, K. & HANDE K.R. (1987) In vitro pharmacodynamic evaluation of VP16-213: implications for chemotherapy. *Cancer Chemother. Pharmacol.*, **19,** 246–249.

WOOD, E.R. & ERNSHAW, W.C. (1990) Mitotic chromatin condensation *in vitro* using somatic cell extracts and nuclei with variable levels of endogenous topoisomerase II. *J. Cell Biol.*, **111,** 2839–2850.

ZINI, N., MARTELLI, A.M., SABATELLI, P., SANTI, S., NEGRI, C., ASTALDI RICHOTTI, G.C.B., & MARALDI, N.M. (1992). The 180-kDa isoform topoisomerase II is localized in the nucleolus and belongs to the structural elements of the nucleolar remnant. *Exp. Cell Res.*, **20,** 460–466.