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Immunocytochemical detection of DNA topoisomerase type II in primary breast carcinomas: correlation with clinico-pathological features

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Abstract. DNA topoisomerase type II (DT–II) is a major component of interphase nuclear matrix fractions, present in S-phase of the cell cycle. A series of 80 carcinomatous breast surgical samples was evaluated by immunohistochemistry, using a polyclonal antibody in a comparison with Ki–67 antiserum. A correlation with clinico-pathological data was also performed. Infiltrating ductal and lobular carcinomas constantly express DT–II with varying intensity of nuclear staining; a similar immunohistochemical pattern is observed with Ki–67. A frequent co-expression of DT–II and Ki–67 is encountered with double immunostaining; accordingly to these data, a linear relationship is evident when linear regression is employed. In addition, significant relationships between DT–II values and tumour size, histological grade and node involvement are shown, while an inverse correlation is appreciable between DT–II and oestrogen receptors and progesterone receptors. DT–II may be considered to be an additional operational marker for the proliferating fraction of cells in breast carcinomas.

Key words: DNA topoisomerase type II – Cell proliferation – Breast carcinoma – Immunohistochemistry

Introduction

DNA topoisomerases are enzymes that control and modify the topological state of DNA; these enzymes have been classified into two types (Liu 1983; Wang 1985; Potmesil et al. 1988). Type I induces transient DNA single-strand breaks, whereas type II introduces transient breaks and passes a double-strand DNA segment through the break (Potmesil et al. 1988). DNA type II topoisomerase (DT–II) also catenate and decatenate closed circular duplex DNA, being implicated in several aspects of DNA metabolism and structure including replication and transcription (Luchnik et al. 1982; Harland et al. 1983; Kaguni and Kornberg, 1984).

It has been reported that DT–II is a major component of interphase nuclear matrix fractions (Berrios et al. 1985); moreover, this enzyme is present in all normal and transformed cells in S-phase with a lack of detectable activity in non-proliferating elements (Duguet et al. 1983; Tandou et al. 1984). Earlier reports have suggested that DT–II activity correlates with cell proliferation (Duguet et al. 1983; Sullivan et al. 1986; Bodley et al. 1987; Chow and Ross 1987; Hsiang et al. 1988), although such a correlation has not been found elsewhere (Tricoli et al. 1985). However, all these studies relied solely upon activity assays, suggesting caution in their interpretation since they might not reflect changes in the total amount of enzyme. Furthermore, Heck and Earnshaw (1986) have shown that DT–II is present in stoichiometric amounts in nuclei of proliferating cells, but this enzyme is rapidly lost when cells cease dividing.

Immunofluorescent detection of DT–II has been reported in the nucleus of proliferating lymphocytes in culture with a significant correspondence with immunoblotting data (Heck and Earnshaw 1986). Recently overexpression of this enzyme has been reported in 36% of node-negative primary breast tumour specimens by a semiquantitative Western blot procedure (Tandon et al. 1991); in addition these data were significantly correlated with a high percentage S-phase fraction and Ki–67 score (Tandon et al. 1991).

In the present study we have performed an immunocytochemical investigation on primary breast carcinomas in an attempt to verify whether DT–II may be considered to be an additional aid in evaluating the rate of cell proliferation. The possible relationships between DT–II immunostaining, Ki–67 expression and clinico-pathological features in the same cases were also analysed.

Materials and methods

In the period 1990–1992, 80 carcinomatous breast surgical samples were collected from female patients (age range 29–85 years; median

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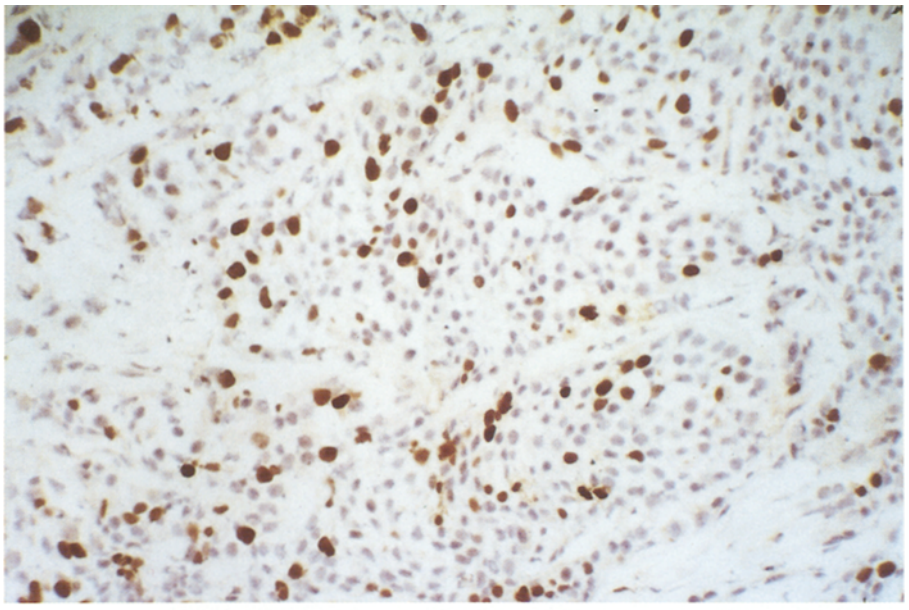

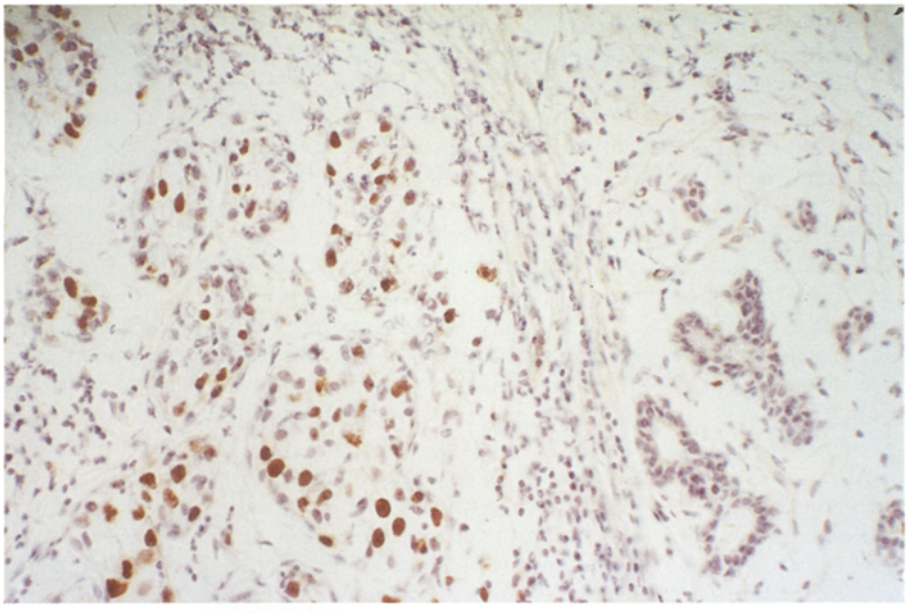

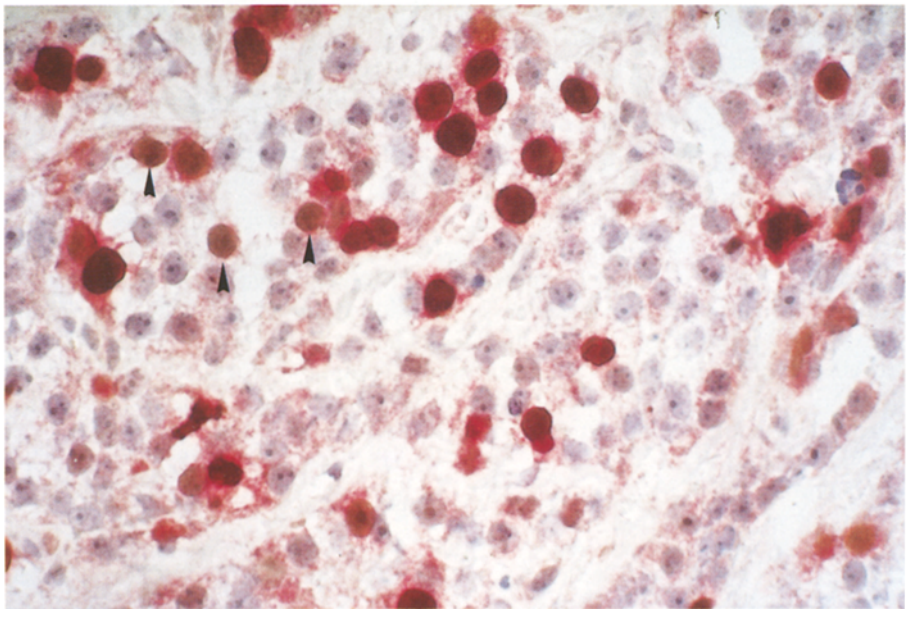

Fig. 1. Infiltrating ductal carcinoma: an intense nuclear positivity for DNA topoisomerase type II is evident in numerous neoplastic elements. Immunoperoxidase with Mayer's haemalum nuclear counterstain, ×150

Fig. 2. Infiltrating lobular carcinoma: some nuclei of neoplastic cells are strongly stained with DNA topoisomerace type II; note the unstained surrounding acini, Immunoperoxidase with Mayer's haemalum counterstain, ×150

Fig. 3. Infiltrating ductal carcinoma: a mixture of brown DNA topoisomerase type II, and red (Ki–67) staining is encountered in some cells; occasionally isolated elements *(arrowheads)* show an exclusive reactivity for DT–II. Immunoperoxidase and alkaline phosphatase with Mayers's haemalum counterstain, ×420

56). Of these, 66 cases were represented by ductal infiltrating carcinomas (18 grade I, 23 grade II, 25 grade III), while 14 cases were diagnosed as lobular infiltrating carcinomas. For all cases the post-surgical stage (pTNM) was available and infiltrating ductal carcinomas were graded according to the method of Bloom and Richardson (1957). Specimens of normal breast tissue adjacent to carcinomas were also tested as controls.

In all breast samples the fixation was omitted and the specimen was frozen in isopentane cooled and liquid nitrogen and stored at −70° C until the time of assay. Sections 5 μm thick were obtained by a Leitz 1720 C digital cryostat and mounted on poly-L-lysine coated glass slides. Parallel sections were treated for 10–15 min in 3.7% phosphate buffered saline formalin solution prior to immunostaining for rabbit anti-human DT–II (Cambridge Research Biochemicals, USA, diluted to 1:200), Ki–67 (Dako, Denmark, at a ditution of 1:100), oestrogen and progesterone receptors (ER, PgR; Abbott, USA). 3–3′ diaminobenzidine tetrahydrochloride was utilized as chromogen and a slight nuclear counterstain was performed by Mayer's haematoxylin. In addition, in order to demonstrate DT–II and Ki–67 on the same section (using both peroxidase and alkaline phosphatase as labels), the two antisera were mixed so as to give the final concentrations found to be appropriate for the individual reagents in preliminary single antigen staining (Mason and Sammons 1978).

Scoring of DT–II and Ki–67 immunostaining was performed by counting a minimum of 1000 cells in multiple random fields, using a high-power (40×) objective with a grid screen; the relationship between the number of cells with DT–II expression and the number of stained elements with Ki–67 was estimated by the linear regression test and a correlation coefficient (r) was calculated. Chi-square analysis was applied in order to evaluate the relationship between DT–II reactivity and immunocytochemical expression of ER and PgR; the same statistical test was also utilized to compare DT–II immunostaining with other categorized clinico-pathological variables such as tumour size, histological grade and nodal status.

Results

All ductal and lobular infiltrating carcinomas showed an evident nuclear reactivity for DT–II, while no cytoplasmatic staining was noted (Figs. 1, 2). A regional variation in the number of cells stained by DT–II was sometimes seen within a single carcinomatous lesion. The rate of stained cells ranged from 3 to 75%, with a median value of 15%; this value was used to discriminate between low and high DT–II immunoexpression.

In normal breast tissue, adjacent to carcinomas, single reactive elements were occasionally observed.

The Ki–67 immunostaining exhibited a pattern similar to that of DT–II, with the proportion of stained cells ranging from 2 to 70% with a median value of 11.5%.

When double immunostaining was performed on the same section, an evident mixture of brown (DT–II) and red (Ki–67) products was appreciated in the nucleus of the great majority of stained neoplastic elements (Fig. 3); in addition, some cells showed a brown nuclear immunostaining due exclusively to DT–II (Fig. 3). When the linear regression test was performed, a significant linear relationship between DT–II and Ki–67 values was encountered ($r=0.882$) (Fig. 4).

Immunostaining of ER and PgR was very heterogenous, the percentage of positive cells running from 0 to 80%; ER and PgR expression was recorded as positive when at least 10% positive cells were encountered throughout the whole tumour area.

The chi-square test showed significant relationships between DT–II values and tumour size, histological

Table 1. DNA topoisomerase type II (DT-II) immunoexpression in 80 primary breast carcinomas in relation to clinico-pathological characteristics

	DT-II (0–15)		DT-II >15		P
All tumours		41 (51.25%)		39 (48.75%)	
Tumour size					<0.02
T_1	28 (35%)		15 (18.75%)		
T_2	12 (15%)		19 (23.75%)		
T_3	1 (1.25%)		5 (6.25%)		
Histology					NS
Infiltrating ductal (total)	32 (40%)		34 (42.5%)		
Grade I		13 (19.69%)		5 (7.57%)	<0.002
Grade II		14 (21.21%)		9 (13.63%)	
Grade III		5 (7.57%)		20 (30.30%)	
Infiltrating lobular	9 (11.25%)		5 (6.25%)		
Axillary nodes					
Negative	30 (37.5%)		18 (22.5%)		<0.03
Positive (total)	11 (13.75%)		21 (26.25%)		
1–3 Nodes		6 (18.75%)		7 (21.875%)	NS
>3 Nodes		5 (15.625%)		14 (43.75%)	
Receptor status					
ER positive	34 (42.5%)		8 (10%)		<0.001
ER negative	7 (8.75%)		31 (38.75%)		
PgR positive	29 (36.25%)		9 (11.25%)		<0.001
PgR negative	12 (15%)		30 (37.5%)		

NS, not significant; ER, oestrogen receptor; PgR, progesterone receptor

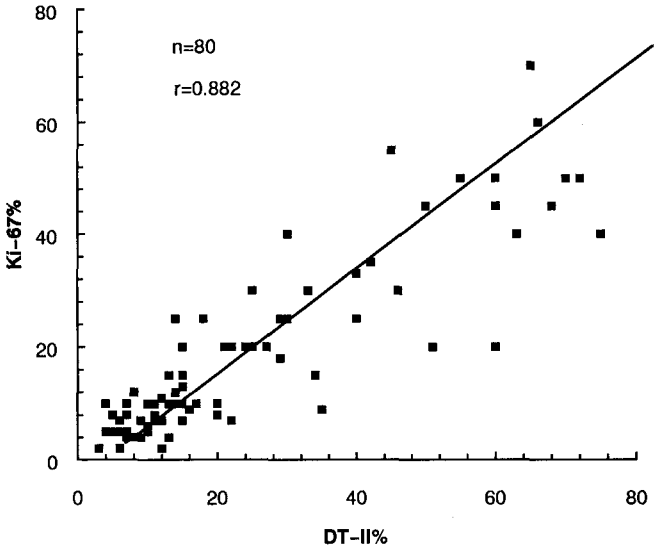

Fig. 4. There is a linear relationship between the number of cells with DNA topoisomerase type (DT–II) expression and the number staining with Ki–67 in primary breast infiltrating carcinomas

grade and axillary lymph node involvement, respectively (Table 1). A significant inverse correlation was noted between DT–II and ER and PgR (Table 1).

Discussion

We have demonstrated that infiltrating ductal and lobular carcinomas constantly express DT–II, although the immunohistochemical distribution pattern of this enzyme was heterogeneous within the same tumour and also among different breast neoplasms; moreover, a similar immunostaining was observed with Ki–67 on parallel sections. These data are strongly supported by the frequent co-expression of DT–II and Ki–67 by double immunostaining in same neoplastic elements. In addition, there is a linear relationship between the number of cells showing DT–II and the number showing Ki–67 immunoreactivity in breast carcinomas, supporting the notion that DT–II immunoreactivity may be used as an operational marker for cell proliferation. However, in order to explain the correlation between DT–II and Ki–67, interesting similarities between Ki–67 antigen and DT–II have been claimed (Verheijen et al. 1989); nevertheless by methods of protein extraction followed by electrophoresis on SDS-polyacrylamide gels, it has been found that the Ki–67 antibody recognized a huge protein doublet of M_r 345 and 395 kDa (Gerdes et al. 1991), whereas the DT–II antibody detected a 175–180 kDa band (Smith and Makinson 1989). In addition, utilizing a cDNA clone it has been confirmed that the chromosomal localization of the Ki–67 gene is 10q25 (ter) (Fonatsch et al. 1991), while DT–II genes have been mapped to chromosomes 3 and 17 (Tan et al. 1992).

Immunohistological methods of assessing cell proliferation have particular advantages over other techniques because of the maintenance of cellular and tissue architecture, the relative simplicity of the methodology and the rapidity of results; neither in vivo nor in vitro labelling is required and the use of radioactivity is avoided (Hall and Levison 1990; Hall and Woods 1990). Immunohistology would be even more useful if available antibodies to cell-cycle-related antigens were applicable to neoplastic tissues. In infiltrating breast carcinomas, a number of studies have looked at the relationship between the growth fraction, as measured by the only widely used antibody that recognizes a cell-cycle-related antigen, Ki–67, and various clinico-pathological features such as tumour diameter, histological grade, lymph node involvement and ER and PgRs (Gerdes et al. 1986, 1987; Barnard et al. 1987; Lelle' et al. 1987; Wrba et al. 1988; Bouzobar et al. 1989; Raymond and Leong 1989; Marchetti et al. 1990). In addition, in our cases, the DT–II immunoexpression shows a direct relationship with tumour size ($P<0.02$), node status ($P<0.03$) and histological grade ($P<0.002$); moreover, no statistical differences are encountered when the histological tumour type and the number of positive lymph nodes are considered. Finally, a significant inverse correlation has been appreciated between DT–II immunostaining and ER ($P<0.001$) or PgR ($P<0.001$).

The possibility of utilizing DT–II immunoexpression as a feature of drug resistance has been reported elsewhere (Tewey et al. 1983; Nelson et al. 1984; Yang et al. 1985; Potmesil 1988; Liu 1989); in fact, there is ample evidence identifying DT–II as the intracellular target of numerous anticancer drugs (Tewey et al. 1983; Nelson et al. 1984; Yang et al. 1985; Potmesil 1988; Liu 1989). In particular, results are consistent with the interpretation that low levels of DNA damage caused by Adriamycin and other drugs in chronic lymphocytic leukaemia and in normal lymphocytes are due to extremely low levels of DT–II (Potmesil et al. 1988). In human breast cancer cells, an increase in DT–II content has been found in a clonogenic cell subpopulation recruited by a cytotoxic drug (VP 16) under oestrogen enhancement (Epstein et al. 1989). In the same study, it has been shown that the increase in DT–II is localized to an activated G1 phase cell subset. In our view immunocytochemical detection of DT–II in breast carcinomas gives an advantage in comparison with other proliferation markers since some information about drug resistance mechanisms may be determined. Further clinical studies are needed in order to evaluate a potential dual role for DT–II in neoplastic pathology.

References

Barnard NJ, Hall PA, Lemoine NR, Kadar N (1987) Proliferative index in breast carcinoma determined in situ by Ki–67 immunostaining and its relationship to clinical and pathological variables. J Pathol 152:287–295

Berrios M, Osheroff N, Fisher PA (1985) In situ localization of DNA topoisomerase II, a major polypeptide component of the *Drosophila* nuclear matrix fraction. Proc Natl Acad Sci USA 82:4142–4146

Bloom HJG, Richardson WW (1957) Histological grading and prognosis in breast cancer. Br J Cancer 11:359–377

Bodley AL, Wu HY, Liu LF (1987) Regulation of DNA topoisomerases during cellular differentiation. Monogr Natl Cancer Inst 4:31–35

Bouzubar N, Walker KJ, Griffiths K (1989) Ki-67 immunostaining in primary breast cancer: pathological clinical associations. Br J Cancer 59:943–947

Chow KC, Ross WE (1987) Topoisomerase-specific drug sensitivity in relation to cell cycle progression. Mol Cell Biol 7:3119–3123

Duguet M, Lavenot C, Harper H, Mirambeau G, De Recondo AM (1983) DNA topoisomerases from rat liver: physiological variations. Nucleic Acids Res 11:1059–1075

Epstein RJ, Smith PJ, Watson JV, Waters C, Bleehen NM (1989) Oestrogen potentiates topoisomerase II mediated cytotoxicity in an activated subpopulation of human breast cancer cells: implications for cytotoxic drug resistance in solid tumours. Int J Cancer 44:501–505

Fonatsch C, Duchrow M, Rieder H (1991) Assignment of the human Ki-67 gene (MK 167) to 10q25–qter. Genomics 11:476–477

Gerdes J, Lelle RJ, Pickartz H (1986) Growth fraction in breast cancers determined in situ with monoclonal antibody Ki-67. J Clin Pathol 39:977–980

Gerdes J, Pickartz H, Brotherton J, Hammerstein J, Weitzel H, Stein H (1987) Growth fractions and estrogen receptors in human breast cancers as determined in situ with monoclonal antibodies. Am J Pathol 129:486–492

Gerdes J, Li L, Schlueter C, Duchrow M, Wohlenberg C, Gerlach C, Stahmer I, Kloth S, Brandt E, Flad HD (1991) Immunobiochemical and molecular biologic characterization of the cell proliferation-associated nuclear antigen that is defined by monoclonal antibody Ki-67. Am J Pathol 138:867–873

Hall PA, Levison DA (1990) Assessment of cell proliferation in histological material. J Clin Pathol 43:184–192

Hall PA, Woods AL (1990) Immunohistological markers of cell proliferation: achievements, problems and prospects. Cell Tissue Kinet 23:531–549

Harland RM, Weintraub H, McKnight SL (1983) Transcription of DNA injected into *Xenopus* oocytes is influenced by template topology. Nature 302:38–43

Heck MMS, Earnshaw WC (1986) Topoisomerase II: a specific marker for cell proliferation. J Cell Biol 103:2569–2581

Hsiang Y, Wu HY, Liu LF (1988) Proliferation-dependent regulation of DNA topoisomerase II in cultured human cells. Cancer Res 48:3230–3235

Kaguni JM, Kornberg A (1984) Replication initiated at the origin (ori C) of the *E. coli* chromosome reconstituted with purified enzymes. Cell 38:183–190

Lelle' RJ, Heidenreich W, Stauch G, Gerdes J (1987) The correlation of growth fraction with histologic grading and lymph node status in human mammary carcinoma. Cancer 59:83–88

Liu LF (1983) DNA topoisomerases: enzymes that catalyse the breaking and rejoining of DNA. CRC Crit Rev Biochem 15:1–24

Liu LF (1989) DNA topoisomerase poisons as antitumor drugs. Annu Rev Biochem 58:351–375

Luchnik AN, Bakayev VV, Zbarsky IB, Georgief GP (1982) Elastic torsional strain in DNA within a fraction of SV40 minichromosomes: relation to transcriptionally active chromatin. EMBO J 1:1353–1358

Marchetti E, Querzoli P, Marzola A, Bagni A, Ferretti S, Fabris G, Nenci I (1990) Assessment of proliferative rate of breast cancer by Ki-67 monoclonal antibody. Mod Pathol 3:31–35

Mason DY, Sammons R (1978) Alkaline phosphatase and peroxidase for double immunoenzymatic labelling of cellular constituents. J Clin Pathol 31:454–460

Nelson EM, Tewey KM, Liu LF (1984) Mechanism of antitumor drug action: poisoning of mammalian DNA topoisomerase II on DNA by 4′-(9-acridinylamino)-methanesulfon-*m*-anisidine. Proc Natl Acad Sci USA 81:1361–1365

Potmesil M (1988) DNA topoisomerase II as intracellular target in anthracycline treatment of cancer. In: Lown JW (ed) Anthracyclines and anthracenedione-based anticancer agents. Elsevier New York, pp –

Potmesil M, Hsiang YH, Liu LF, Bank B, Grossberg H, Kirschenbaum S, Forlenzar T, Penziner A, Kanganis D, Knowles D, Traganos F, Silber R (1988) Resistance of human leukemic and normal lymphocytes to drug-induced DNA cleavage and low levels of DNA topoisomerase II. Cancer Res 48:3537–3543

Raymond WA, Leong AS-Y (1989) The relationship between growth fractions and oestrogen receptors in human breast carcinoma, as determined by immunohistochemical staining. J Pathol 158:203–211

Smith PJ, Makinson TA (1989) Cellular consequences of overproduction of DNA topoisomerase II in an ataxia-telangiectasia cell line. Cancer Res 49:1118–1124

Sullivan DM, Glisson BS, Hodges PK, Smallwood-Kentro S, Ross WE (1986) Proliferation dependence of topoisomerase II mediated drug action. Biochemistry 25:2248–2256

Tan KB, Dorman TE, Falls KM, Chung TDY, Mirabelli CK, Crooke ST, Mao J (1992) Topoisomerase II alfa and topoisomerase II beta genes: characterization and mapping to human chromosomes 17 and 3, respectively. Cancer Res 53:231–234

Tandon AK, Hilsenbeck SG, Clark GM, Allred DC, Latham MD, Ross WE, McGuire WL (1991) Significance of topoisomerase II in clinical breast cancer. Proc Am Assoc Cancer Res 32:350

Tandou G, Mirambeau G, Lavenot G, Garabedian A der, Vermeersch J, Duguet M (1984) DNA topoisomerase activities in concanavalin A-stimulated lymphocytes. FEBS Lett 176:431–435

Tewey KM, Rowe TC, Yang L, Halligan BD, Liu LF (1983) Adriamycin-induced DNA damage mediated by mammalian DNA topoisomerase II. Science 226:466–468

Tricoli JV, Sahai BM, McCormick PJ, Sarlinski SJ, Bertram JS, Kowalski D (1985) DNA topoisomerase I and II activities during cell proliferation and the cell cycle in cultured mouse embryo fibroblast (C3H10T1/2) cells. Exp Cell Res 158:1–14

Verheijen R, Kuijpers HJH, Van Driel R (1989) Ki-67 detects a nuclear matrix-associated proliferation-related antigen. II. Localization in mitotic cells and association with chromosomes. J Cell Sci 92:531–540

Wang JC (1985) DNA topoisomerases. Annu Rev Biochem 54:665–697

Wrba F, Reiner A, Markis-Ritzinger E, Holzner JH, Reiner G, Spona J (1988) Prognostic significance of immunohistochemical parameters in breast carcinomas. Pathol Res Pract 183:277–283

Yang L, Rowe TC, Liu LF (1985) Identification of DNA topoisomerase II as an intracellular target of antitumor epipodophyllotoxins in Simian Virus 40-infected monkey cells. Cancer Res 45:5872–5876