Differential expression of the topoisomerase II\(\alpha\) and \(\beta\) genes in human breast cancers

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Summary Topoisomerase II is a key target for several anti-cancer drugs used for breast cancer therapy, including doxorubicin, epirubicin and mitoxantrone. Two isoforms of topoisomerase II (\(\alpha\) and \(\beta\)) have been described in human cells which differ in their subcellular localisation, biochemical properties and susceptibility to inhibition by anti-cancer drugs. The relative level of expression of the \(\alpha\) and \(\beta\) isoforms may contribute to the degree of tumour responsiveness to different chemotherapeutic agents. To assess the relationship between expression of topoisomerase II isoforms and established prognostic factors and pathological variables, 56 primary breast tumour samples were studied. The expression of the two topoisomerase II genes was apparently not co-ordinately regulated in these tissue samples. There was no relationship between any of the commonly used pathological variables (tumour size, lymph node status, S-phase fraction (SPF)) and the level of expression of topoisomerase II\(\beta\) mRNA. However, high topoisomerase II\(\alpha\) gene expression was significantly associated with a high SPF (sign-rank test: \(P=0.01\)). Moreover, the ratio of mRNA levels for topoisomerase II\(\alpha\) and \(\beta\) showed a stronger relationship to SPF (median ratio 0.62 for tumours with SPF < 10, and 1.64 for SPF > 10; \(P=0.0021\), sign-rank test). As expected from previous studies, an SPF > 10 was associated with poor overall survival (\(P=0.01\)). Immunohistochemical analysis revealed that topoisomerase II\(\beta\) was widely distributed (>90% positive tumour cells), but that topoisomerase II\(\alpha\) expression was less widely expressed, with a pattern of expression similar to that of the proliferation-dependent antigen recognised by Ki67. Because topoisomerase II gene expression showed a log-normal distribution, log-transformed data were used in multivariate analysis of relapse-free survival. This showed that lymph node status and topoisomerase II\(\beta\) mRNA expression were the only significant survival factors (\(P=0.001\) and 0.05, respectively, with relative risks of 1.3 and 1.8). These results indicate that topoisomerase II\(\alpha\), but not \(\beta\), expression is dependent upon cellular proliferation status, but that the more widely expressed topoisomerase II\(\beta\) protein may play a significant role as a target for anti-tumour therapy.

Keywords: topoisomerase II\(\alpha\); topoisomerase II\(\beta\); breast cancer; S-phase fraction

There is an extensive body of work detailing the potential value of prognostic markers in breast cancer. The major objective of such studies is to separate patients into low- and high-risk categories permitting effort to be concentrated on those patients in the latter category. Such an approach has been found useful in determining the benefits of chemotherapy, such as the combination of cyclophosphamide, methotrexate and 5-fluorouracil (CMF), in patients with affected regional lymph nodes (Early Breast Cancer Trialsists' Group, 1992). The standard prognostic indices that have been assessed in node-negative breast cancer are tumour size, histological classification, nuclear grade, oestrogen and progesterone receptor status, DNA ploidy and S-phase fraction (SPF; reviewed by McGuire and Clark, 1992). There is also evidence that cathepsin D levels, epidermal growth factor receptor (EGFR) status and the presence of the HER/\(\text{neu}\) oncogene may be of some prognostic value (reviewed by Gasparini et al., 1993). Recently, expression of p53 protein has been shown to be associated with a high tumour proliferation rate, early disease recurrence and early death in node-negative breast cancer patients (Allred et al., 1993). In addition to the identification of prognostic factors, there is a clear need for predictive markers that will permit both the development of more appropriate adjuvant chemotherapy and the selection of those patients most likely to respond to a particular drug regimen. A potential prognostic indicator that could also be predictive of response to chemotherapy is the level of expression of topoisomerase II. This essential nuclear enzyme is the primary cellular target for several of the most effective anti-tumour agents including doxorubicin, etoposide, epirubicin and mitoxantrone (reviewed by Smith, 1990; Osheroff et al., 1991; Beck et al., 1993; Pommier, 1993; Watt and Hickson, 1994). Topoisomerases catalyse the interconversion of topological isomers of DNA. The type II topoisomerases, such as topoisomerase II, act via the introduction of a transient double-stranded break in one segment of a DNA molecule through which a second DNA duplex is passed before religation of the break. In mammalian cells, a role for topoisomerase II has been suggested in DNA replication, recombination and possibly transcription, as well as in mitotic chromosome condensation and segregation (reviewed by Osheroff et al., 1991; Wang, 1985; Watt and Hickson, 1994). Topoisomerase II is also a structural component of the interphase nucleus, possibly anchoring looped domains of chromatin to the nuclear scaffold or matrix (Earnshaw et al., 1985; reviewed by Roberge and Gasser, 1992).

Topoisomerase II protein levels are markedly higher in exponentially growing than in quiescent cell lines in tissue culture, and can be down-regulated by growth of cells at high density or in serum-free conditions (Hsiang et al., 1988). Thus, topoisomerase II may be regarded as a marker of cell proliferation. Moreover, cells induced to differentiate show progressively reduced levels of topoisomerase II activity (Constantinou et al., 1989; Zwelling et al., 1990).

Two distinct isoforms of topoisomerase II exist in human cells, termed \(\alpha\) (170 kDa form) and \(\beta\) (180 kDa form), which differ not only in molecular weight but also in their patterns of expression and their apparent sensitivity to anti-neoplastic drugs (Drake et al., 1989; Chung et al., 1989; Woessner et al., 1990; Jenkins et al., 1992; Austin et al., 1993). In cell lines, the expression of the \(\alpha\) isoform has been shown to be strictly proliferation dependent, whereas the \(\beta\) isoform is present in...
both dividing and non-dividing cells (Woessner et al., 1991). However, this pattern of expression may not be maintained in vivo, since it has been reported that lymphocytes induced to proliferate by exposure to proliferating human antigen (PHA) show increased expression of both the $\alpha$ and $\beta$ isoforms (Kaufmann et al., 1994; Prosperi et al., 1994).

Work on cell lines has shown that the levels of the topoisomerase II$\alpha$ and/or $\beta$ mRNAs may decrease in cells made resistant to topoisomerase II inhibitory drugs, and that such changes may account for the decreased levels of protein found in cell lines expressing the so-called 'atypical' multidrug resistant phenotype (reviewed by Beck et al., 1993; Pommier, 1993). There are documented decreases in both topoisomerase II$\alpha$ and topoisomerase II$\beta$ protein in such resistant cell lines. Several studies have shown a correlation between topoisomerase II protein levels and sensitivity of cells to these drugs, with elevated levels conferring relative drug sensitivity and low levels conferring resistance (Davies et al., 1988; Potmesil et al., 1988; Webb et al., 1991; reviewed by Beck et al., 1993). Similarly, teratoma cell lines with a greater sensitivity than bladder cell lines to topoisomerase II poisons have been shown to express a correspondingly higher level of topoisomerase II protein (Fry et al., 1991).

Topoisomerase II inhibitors such as doxorubicin, epirubicin and mitoxantrone are widely used in therapy for breast cancer. The aim of this study was to quantify the level of expression of the two topoisomerase II isoforms in breast tumour biopsies and to investigate whether a relationship exists between the level of topoisomerase II gene protein expression and the established prognostic indicators for patient survival.

Materials and methods

Preparation of mRNA

Tumour samples were obtained from patients undergoing breast surgery at the John Radcliffe Hospital, Oxford, UK, and were histologically confirmed as intraductal carcinomas. Samples were snap frozen and stored in liquid nitrogen before extraction of total cellular RNA by the method of Chomczynski and Sacchi, (1987). RNA concentration was quantified by measurement of optical density at 260 nm. Integrity of RNA was assessed by running samples on 1% agarose gels followed by staining with ethidium bromide.

Ribonuclease protection assays

Ribonuclease protection assays were carried out as described by Jenkins et al. (1992). Topoisomerase II$\alpha$ and $\beta$ antisense RNA probes were prepared as described previously (Davies et al., 1993). The topoisomerase II$\alpha$- and II$\beta$-specific probes generated 215 bp and 228/292 bp (two splice variants termed II$\alpha$-1 and II$\alpha$-2) protected fragments respectively. In each reaction an internal loading control of an antisense transcript to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used (producing a 120 bp protected fragment). Quantification of image intensities and autoradiograms was performed using a Bio-Image analyser (MiliGen/BioSearch).

DNA flow cytometry

Nuclei were extracted from a 50 $\mu$m paraffin-embedded tissue section for each tumour as described previously (Camplejohn et al., 1989). Briefly, each section was dewaxed, rehydrated and was treated for 30 min at 37°C with pepsin, pH 1.5. Debris was removed by filtration through a 35 $\mu$m pore size nylon gauze and the nuclei were stained with a DNA-specific dye DAPI at a concentration of 1 $\mu$g ml$^{-1}$. DNA content in at least 10$^5$ nuclei was measured on a Becton-Dickinson FACSort FACS analyser. DNA aneuploidy was recorded only if two distinct G1 peaks were evident. S-phase fraction (SPF) for diploid tumours was calculated by the method of Baisch et al. (1975), and by a modification of this method for aneuploid tumours as described previously (Camplejohn et al., 1989).

Immunohistochemistry staining for topoisomerase II$\alpha$ and $\beta$

Breast tumour biopsies were obtained fresh after surgery and representative areas were cut and snap frozen. Cryostat sections (8 $\mu$m) were cut and mounted onto poly-L-lysine-coated glass slides. After drying for 0.5 to 8 h, the sections were fixed in phosphate-buffered saline containing 3.7% formalin for 15 min at room temperature, and then immediately stained using the immunoperoxidase 'Duet' kit (Dako). The antibodies used were as follows: the topoisomerase II$\alpha$-specific rabbit polyclonal antibody termed CRB (Cambridge Research Biochemicals) which has been raised in previous studies (Smith and Makinson, 1989; Wells et al., 1994). The topoisomerase II$\beta$-specific mouse monoclonal antibody designated 3H10 was kindly supplied by Dr A Kikuchi. This antibody was raised to a peptide in the C-terminal domain of mouse topoisomerase II$\beta$ protein and recognises a single 180 kDa protein in human cell extracts (A Kikuchi, personal communication; H Turley, in preparation).

Moreover, the extensive down-regulation of topoisomerase II$\beta$ protein in a mitoxantrone-resistant CEM cell line, compared with its parental CEM cell line, is detected by this antibody (unpublished data). The Ki67 antibody recognises an antigen expressed exclusively in proliferating cells and has been used previously as a marker of proliferation in immunohistochemical studies (Gerdes et al., 1984; Verheijen, 1989; Gerdes et al., 1991). Staining with Ki67 was performed after fixing the sections in acetone at room temperature for 10 min and drying.

The staining was graded by the percentage of tumour cells expressing topoisomerase II$\alpha$ as follows: grade 1 (5%), grade 2 (5–25%), grade 3 (25–50%) and grade 4 (75%). This also applied to staining with the Ki67 antibody. Since nearly all tumour cells stained for topoisomerase II$\beta$, intensity alone was graded as 1+, 2+ or 3+.

Hormone receptors

Oestrogen and EGF receptors were measured by ligand binding on tumour cytosols and membranes respectively, as described previously (Harris et al., 1989; Smith et al., 1993).

Patients' characteristics

Patients were treated by wide local excision or simple mastectomy and node sampling was performed in all cases. Post-operative radiation therapy was given to the breast after local excision and to the axilla if lymph nodes were positive. Adjuvant tamoxifen (20 mg daily) was given to all women aged 50 or over, whereas six courses of adjuvant CMF therapy were given to all node-positive patients under 50. Node-negative patients under 50, with tumours larger than 5 cm, or those with vascular invasion, were also treated with CMF. The patients were seen at 3 month intervals for the first 2 years, 6 monthly during the third year and once yearly thereafter. Patient variables are shown in Table I. Survival analyses was by the Kaplan–Meier method, with Cox multivariate analyses.

Results

Expression of topoisomerase II$\alpha$ and $\beta$ mRNAs

Previous studies have shown that acquired resistance to topoisomerase II inhibitors can be correlated with down-regulation of topoisomerase II gene expression. Conversely, overexpression of topoisomerase II confers relative drug sensitivity in cell lines (Davies et al., 1988; Potmesil et al., 1988; Webb et al., 1991; reviewed by Beck et al., 1993). Ribonuclease protection assays were used to quantify the level of expression of the topoisomerase II$\alpha$ and $\beta$ mRNAs in
56 samples extracted from patients with ductal carcinoma of the breast. The single topoisomerase IIα mRNA and the two alternatively spliced topoisomerase IIβ (β-1 and β-2) mRNAs were detected in all tumour samples studied. There was considerable variability between tumours in the level of expression of the two topoisomerase II genes. The data from a representative RNase protection assay is presented in Figure 1. The levels of topoisomerase II mRNAs were quantified by densitometric scanning of early exposure autoradiograms within the linear range for radiographic film. The results were then standardised by comparison with the level of an internal control of the housekeeping gene, GAPDH, with the median value defined as 1. Values ranged from 0.44 to 0.08 (approximately 500-fold range) for topoisomerase IIα mRNA and 16.5 to 0.05 for topoisomerase IIβ (an approximately 300-fold range).

There was no correlation ($P>0.05$) between the relative levels of the topoisomerase IIα and β mRNAs in individual tumours (Figure 2). Thus, in some samples with low levels of topoisomerase IIα mRNA, there were equivalently low levels of topoisomerase IIβ mRNA (such as sample 9; Figure 1), whereas in other cases with low topoisomerase IIα mRNA expression, the level of topoisomerase IIβ mRNA was substantially higher (sample 6; Figure 1). The relative level of the β-1 and β-2 mRNAs was generally constant in each sample.

**Relation to topoisomerase II mRNA to SPF and ploidy**

The tumours were studied with respect to the relationship between topoisomerase II mRNA expression and SPF. Those cases with a high SPF (defined as being >10%) showed significantly higher topoisomerase IIα mRNA that those with a low SPF (<10%) (median 1.47 and 0.42 respectively, using the Mann–Whitney U-test for non-parametric samples; $P=0.01$ level). Because the data was log-normally distributed, the Spearman rank correlation coefficient for log-topoisomerase IIα vs SPF was performed (Figure 3), and showed a correlation coefficient of 0.33 ($P=0.01$). Using the median densitometric value of 1 to separate the cases on the basis of topoisomerase IIα mRNA content, it was found that SPF was significantly higher in those with topoisomerase IIα values above the median ($P=0.03$; Fisher’s exact test). However, there was no relationship between SPF and the level of the topoisomerase IIβ mRNA or log topoisomerase IIβ mRNA (data not shown). The SPF was more highly related to the ratio of topoisomerase IIα to β mRNA than to topoisomerase IIα mRNA level alone (Figure 4) (SPF < 10, median ratio $x_{\beta}=0.62$; SPF > 10, median ratio $x_{\beta}=1.64$, $P=0.002$ ranked sum test). No correlation was found between the

| Table 1 Patient and tumour characteristics |
|-------------------------------|-------------|
| Variable          | No. of cases |
| Age (years)       |             |
| < 50              | 22          |
| ≥ 50              | 34          |
| Size (cm)         |             |
| < 2               | 15          |
| ≥ 2               | 41          |
| Nodes             |             |
| Negative          | 30          |
| Positive          | 26          |
| SPF (%)           |             |
| < 10              | 33          |
| ≥ 10              | 23          |
| ER (fmol mg⁻¹ protein) |         |
| < 10              | 18          |
| ≥ 10              | 38          |
| EGFR (fmol mg⁻¹ membrane protein) | |
| < 20              | 27          |
| ≥ 20              | 29          |

![Figure 1](image1.png) **Figure 1** RNase protection assays of topoisomerase IIα and β mRNA levels (and a GAPDH internal control) in breast tumour biopsies. Lanes 1 through 17 show RNAs from different tumour samples. The positions of the topoisomerase IIα, β-1, β-2 and GAPDH protected fragments are shown on the left. The lane marked MWM contains molecular weight standards which were run in parallel. The sizes of the standards are shown on the right (in base pairs). Densitometric scanning of autoradiograms was performed when each signal was within the linear range for radiographic film.

![Figure 2](image2.png) **Figure 2** Topoisomerase IIα vs topoisomerase IIβ expression in human primary breast cancers. RNA was quantitated by densitometry after RNase analysis and corrected for GAPDH expression. Log-transformed results are shown.

![Figure 3](image3.png) **Figure 3** Log topoisomerase IIα mRNA expression vs S-phase fraction in primary breast cancers.

![Figure 4](image4.png) **Figure 4** The ratio of topoisomerase IIα to β mRNA vs S-phase fraction in primary breast cancers.
degree of tumour ploidy and the level of expression of either topoisomerase IIα or β mRNA using a Mann-Whitney analysis (data not shown). Ploidy was also compared as a bivariable (diploid/aneuploid) and as a continuous variable, but was not significantly associated with topoisomerase IIα or β mRNA expression.

**Topoisomerase II expression and prognosis**

There was no relationship between topoisomerase IIα mRNA expression, stratified as above or below the median level of 1, and age, nodal status, tumour size or oestrogen and epidermal growth factor receptor levels (data not shown). Similar analyses were performed for topoisomerase IIβ, but the P-values for all of these analyses were above 0.05. Over the 5 year period since this study was initiated, the overall level of patient survival has declined to 75% at 5 years (actuarial analysis). Those patients with an SPF of greater than 10 showed a significant (P=0.014) reduction in survival probability relative to those patients with an SPF of less than 10 (Figure 5). Thus, this group of patients, although relatively small in number, is representative of previously reported associations of SPF with overall survival. In a multivariate analysis of relapse-free survival, lymph node status was the major independent factor (P=0.001, relative risk 1.33, confidence intervals 1.12–1.58). However, upon analysis of many other factors, including age, tumour size, lymph node involvement, SPF and log topoisomerase IIα and β mRNA expression, only expression of topoisomerase IIβ mRNA was of additional prognostic significance (P=0.05, risk of 1.81, confidence interval 1–3.3).

**Topoisomerase II protein expression**

To assess the relationship between expression of mRNA and protein for topoisomerase IIα and β, immunocytochemical analysis was conducted on ten cases with SPF>11 (median 16) and ten cases with SPF<6.5 (median 3.5) using isozyme-specific antisera. Figure 6 shows a representative tumour section stained with anti-topoisomerase IIα and IIβ antibodies. In all cases studied, topoisomerase IIβ protein expression was very widely distributed in both tumour tissue and surrounding stroma. In contrast, a significant level of staining for topoisomerase IIα protein was seen only in a limited proportion of the tumour cells and was absent from the surrounding stroma.

The percentage of tumour cells staining positively for the Ki67 antigen correlated well with the distribution of cells staining positive for topoisomerase IIα protein (P=0.01), but not with the intensity of topoisomerase IIβ staining. Intensity

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**Figure 5** Overall survival in breast cancer patients stratified by S-phase fraction.

**Figure 6** Immunoperoxidase staining of cryostat sections of breast carcinomas biopsies with anti-topoisomerase IIα (a and c) and IIβ (b and d) antibodies. a and b represent a biopsy with an SPF of 17.3% while c and d represent a biopsy with an SPF of 4.5%. Note the near absence of positive staining for topoisomerase IIα (but not IIβ) in the biopsy with the lower SPF.
of staining for topoisomerase IIβ was not related to the percentage of cells staining positively for topoisomerase IIα protein. In all of the cases, the percentage of cells staining positive for topoisomerase IIβ exceeded that staining positive for topoisomerase IIα. The staining intensity and proportion of cells staining positive did not correlate closely with mRNA levels for either isoform.

Discussion

The aim of this study was to determine the expression of topoisomerase II isoforms in clinical samples from patients with breast cancer and to determine whether there was any correlation between expression of either isoform and number of different prognostic markers. In recent years, numerous studies have been carried out to assess the benefit of measuring the SPF (representing the percentage of cells in active DNA synthesis) in breast cancer as a prognostic marker. Most studies have shown an association between high SPF and relapse-free survival. The evidence for a link between tumour ploidy and relapse-free survival has been less clear (reviewed by O'Reilly and Richards, 1992). The majority of the previous studies have looked at both node-negative and node-positive cancers. For example, a study of patients with node-negative breast cancer found that those patients with tumours of greater than 1.0 cm, and with an SPF above 10%, had a 5 year relapse-free survival of 52%, whereas those with tumours with an SPF below 10% had a 5 year relapse-free survival of 78% (for tumours greater than 1 cm) or 96% (tumours less than 1 cm) (O'Reilly et al., 1990). Although other studies have confirmed the link between SPF and disease-free survival, the relative survival difference reported has varied between studies. For example, one study of 396 patients showed only a 10% difference in survival between patients whose tumours had low or high SPF (Fisher et al., 1991). An SPF of 10% as a discriminator has been widely used to distinguish groups of patients and for that reason we used this value in our study. A correlation was found between tumours expressing high topoisomerase IIα mRNA level and high SPF (defined as being above 10%). Some of the samples analysed expressed topoisomerase IIα mRNA levels more than 20 times the median level. Among these were tumours with an SPF above 15%. Moreover, one of the samples is known to show gene amplification at the topoisomerase IIα locus (Smith et al., 1993).

We had thought that there might be an association between topoisomerase IIα gene expression and the degree of ploidy in tumours in view of the role of topoisomerase IIα in chromosome structure and dynamics (reviewed by Wang, 1985; Roberge and Gasser, 1992; Watt and Hickson, 1994). However, there was no correlation between tumour ploidy and expression of either isozyme. Moreover, there was also no correlation between levels of topoisomerase IIα and either hormone receptor status or tumour size. In our study, the levels of the two topoisomerase IIα isoforms varied significantly between different tumours with a maximum 200-fold variation in expression between the highest and lowest expression for each isozyme. Despite this, no pattern was found which might suggest that the topoisomerase IIα and β genes are coordinately regulated in breast tumours. Indeed, Jenkins et al. (1992) have shown that the topoisomerase IIα and β genes are apparently independently regulated in cell lines.

A small study comparing topoisomerase IIα mRNA by dot-blot analysis found that expression was high in nine out of ten tumour samples studied but was detectable in only 50% of adjacent normal tissues (Kim et al., 1991). Levels of topoisomerase IIα expression were also studied in chronic lymphocytic and acute leukaemias by slot-blot analysis with high levels of topoisomerase IIα in acute leukaemias (Gekeler et al., 1992). A study investigating topoisomerase I, topoisomerase II, MDR and glutathione S-transferase-α mRNA expression failed to detect any topoisomerase IIα (presumably α2) mRNA in samples of myeloma cells (Ishikawa et al., 1993). This last study used Northern blotting which is less sensitive than the ribonuclease protection assay used here. Our study, unlike the other investigations, determined the differential expression of the two topoisomerase II α isoforms. D'Andrea et al. (1990) studied eight breast cancers and found a good correlation between expression of Ki67 and topoisomerase IIα. Using an antibody to topoisomerase IIβ, that does not detect the full-sized topoisomerase IIβ protein, these authors found no association between the degree of staining for the topoisomerase IIα and β proteins.

An increase in mRNA may be secondary to amplification of topoisomerase IIα gene. A smaller primary breast cancers found amplification of erbB-2, which is located close to the topoisomerase IIα locus, in 25 cases and coamplification with the topoisomerase IIα gene in three cases (Smith et al., 1993). Amplification of the topoisomerase IIα locus was not found. In the cultured cell line SKBr-3, amplification of erbB-2 was also associated with topoisomerase IIα amplification; this line also showed increased sensitivity to the topoisomerase IIα inhibitors m-AMSA and mitoxantrone. These examples illustrate that an increase in topoisomerase IIα mRNA could reflect genetic changes within a tumour (Keith et al., 1993).

In our multivariate analysis, a high level of topoisomerase IIβ mRNA expression was associated with a higher risk of relapse. However, the significance was borderline and there was no association with other factors that might provide an explanation for this. It might have been expected that a decrease in topoisomerase IIβ mRNA gene expression would be needed to generate a drug-resistant tissue, assuming that this isozyme is a significant target for drugs in vitro. However, if topoisomerase IIβ is relatively drug resistant compared with topoisomerase IIα in vivo, as has been demonstrated in vitro (Drake et al., 1989), it is possible that tumours expressing a high relative level of topoisomerase IIβ might be more resistant to topoisomerase II inhibitors than those with a high level of topoisomerase IIα gene expression. Whether the observed up-regulation of topoisomerase IIβ in some tumours reflects a stress or stromal response associated with a more aggressive cellular phenotype is not clear at this stage. This is currently being assessed in cell lines.

It is clear that the high level of topoisomerase IIα mRNA seen in cell lines is a reflection of proliferation (Woessner et al., 1991). In the tumour biopsies analysed in this study, although topoisomerase IIα expression correlated significantly with SPF, the number of proliferating cells in each tumour was quite low compared with cell lines in vitro. The overall level of topoisomerase IIα mRNA expression did not seem to correlate directly with the proportion of the tumour cells expressing topoisomerase IIα protein, suggesting that topoisomerase IIα levels may also be regulated post-transcriptionally. The intensity of staining for topoisomerase IIα did not correlate with the level of topoisomerase IIβ mRNA, as determined by the ribonuclease protection assay. Thus, we concluded that measurement of mRNA levels for either isoform is unlikely to present a true measure of the overall level of the equivalent protein. This is particularly important to consider since biopsy samples for analysis of topoisomerase IIα mRNA will inevitably include some contaminating stromal tissue that we have shown expresses this isoform. Equally important is the finding that topoisomerase IIα is expressed in only a limited number of proliferating tumour cells. Thus, RNA in situ determinations for this isoform in homogenised tumour biopsies may provide a measure of SPF rather than a true measure of the tumour to tumour variation in expression of the α isoform.

Hellemans et al. (1995) recently published an immunohistochemical study of topoisomerase IIα expression in ductal carcinoma of the breast. In agreement with our data, they observed a highly variable proportion of tumour cells which expressed topoisomerase IIα protein, with a median level of
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Topoisomerase II expression of 14% in tumour cells (with many tumours showing >25% positive cells for topoisomerase IIα). However, Helleman et al. (1995) found a positive correlation between topoisomerase IIβ protein expression and nodal status, tumour size and grade, that we did not observe. Sasaki et al. (1995) reported in their study nor was the β isozyme analysed. What is clear from our study is that topoisomerase IIα/β gene expression varies greatly among different breast tumour biopsies, but that topoisomerase IIβ protein is generally distributed in >90% of all tumour cells, irrespective of their proliferation status. Whether proliferation further enhances topoisomerase IIβ expression was not possible to discern from our study, although it should be noted that the stromal cells within tumour tissue were frequently stained more strongly for topoisomerase IIβ than stromal cells in adjacent normal breast tissue.

Tuccari et al. (1993), using a polyclonal antibody to topoisomerase IIα, found a correlation of topoisomerase expression with Ki67, in agreement with our data. Topoisomerase II enzyme activity has been quantified in one study of biopsies from various tumour types. In this, MacLeod et al. (1994) found that topoisomerase II activity was lower in breast cancers than in several other tumour types, although the identity of the isozymes responsible for this activity was not discerned. There was a wide range of topoisomerase IIα activities, similar to the range of expression seen immunohistochemically.

It is clear that a significant proportion of breast cancers respond to topoisomerase II inhibitors, even when given as a single agent. Considering the low proportion of breast tumour cells in S-phase, or that express high levels of the α isozyme of topoisomerase II, we would suggest that the β isozyme may represent a significant (and possibly the primary) target in vivo for chemotherapeutic agents which target topoisomerase II. Further studies are required to confirm this suggestion. In summary, we would suggest that selection of the subset of patients with tumours expressing a high level of topoisomerase IIα and/or β expression treatment with topoisomerase II inhibitors may improve response rates.

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