Eur J Cancer, Vol. 29A, No. 10, pp. 1469–1475, 1993.
Printed in Great Britain

0964–1947/93 $6.00 + 0.00

Co-amplification of *erb*B2, Topoisomerase II α and Retinoic Acid Receptor α Genes in Breast Cancer and Allelic Loss at Topoisomerase I on Chromosome 20

W.N. Keith, F. Douglas, G.C. Wishart, H.M. McCallum, W.D. George, S.B. Kaye and R. Brown

The DNA topoisomerase enzymes are targets for the cytotoxic effects of a number of anticancer agents termed topoisomerase inhibitors. We have analysed breast cancer biopsy specimens for genetic alterations at and around topoisomerase loci in order to obtain molecular insight into factors which may determine how tumours respond to chemotherapy. We show that of 50 tumours examined, the topoisomerase II α locus is co-amplified in 3 cases out of 6 with *erb*B2 amplification and that amplification can be accompanied by high expression of topoisomerase II α. In our attempts to distinguish amplification from aneuploidy and define the limits of amplification, we also observed co-amplification of the retinoic acid-α receptor with *erb*B2 and topoisomerase II α in the same three samples. At the topoisomerase I locus on chromosome 20, we observed allelic loss in two out of 17 samples. Genetics abberations at topoisomerase loci, therefore, appear to be relatively common in breast cancer.
Eur J Cancer, Vol. 29A, No. 10, pp. 1469–1475, 1993.

INTRODUCTION

A NUMBER of genetic events common to the development of breast cancer have been elucidated through the study of somatic loss of heterozygosity (LOH) [1]. This loss of genetic material often occurs in regions of the chromosome associated with localisation of tumour suppressor genes, the functional deletion of which is required to deregulate normal cell growth during multistage carcinogenesis [1]. The study of restriction fragment length polymorphisisms (RFLP) has identified a number of loci lost in breast cancer and located on chromosomes including 1,3, 11,13,17 and 18 [1]. Another frequent event is amplification of the *erb*B2 oncogene [1, 2]. *erb*B2 is a growth factor receptor with homology to the receptor for epidermal growth factor [2] and there is considerable clinical interest in *erb*B2 as a prognostic factor in breast cancer [3].

Once a tumour has been diagnosed, successful treatment with chemotherapeutic agents is often hampered by intrinsic or acquired resistance to drugs. A number of these anticancer drugs are topoisomerase inhibitors which target the topoisomerase enzymes located in the nucleus [4]. Topoisomerase enzymes catalyse breakage and rejoining of the DNA and are, therefore, involved in many basic cellular processes such as chromosome replication, condensation and segregation as well as DNA repair and transcription [4]. In addition, their role in the maintenance of genome stability [5] may be of particular importance in tumour development. Topoisomerases can be divided into two classes. Type 1 topoisomerases (topoisomerase I) introduce a single nick into the double helix of DNA, whereas type 2 topoisomerases (topoisomerase II) produce a staggered double stranded cleavage [4].

The levels of functional topoisomerase enzymes in cell lines can determine the sensitivity of cells to topoisomerase inhibitors [6]. Thus alteration of topoisomerase genes during carcinogenesis may in part account for the response of tumours to certain anticancer drugs [7]. Both the genes encoding topoisomerase II α and *erb*B2 reside on chromosome 17q [8, 9] and their co-amplification in an adenocarcinoma cell line has recently been reported [7]. High levels of topoisomerase II will sensitise cells to the cytotoxic effects of topoisomerase inhibitors [6]. The frequency of co-amplification of *erb*B2 and topoisomerase II α in adenocarcinomas is, therefore, of importance in designing better chemotherapy regimens and refining prognostic markers in breast cancer.

The retinoic acid receptor alpha protein (RAR α) is a hormone dependent transcriptional transactivator involved in the regulation of differentiation and proliferation [10]. As with topoisomerase II α and *erb*B2, RAR α has been mapped to chromosome 17q 21 [11]. Of particular interest is the association of translocation between chromosomes 17 and 15 in acute promyelocytic leukaemia (APL) which fuses the RAR α gene with a putative DNA binding protein on chromosome 15, namely promyelocytic leukaemia gene (PML) [12]. The PML-RAR α fusion protein is a weaker transactivator than the normal RAR α receptor, and it has been suggested that this may be a contributing event in the leukaemogenesis [12]. Due to the observation that APL is sensitive to treatment with retinoic acid, the abnormal expression of PML-RAR α may be overcome by high levels of hormone [10, 12]. Thus a link between molecular genetics of tumour development and the response of the tumour to therapy has been proposed [12]. Breast cancer cells are also responsive to retinoid treatment [10].

Correspondence to W.N. Keith.
W.N. Keith, F. Douglas, G.C. Wishart, S.B. Kaye and R. Brown are at the CRC Department of Medical Oncology, Beatson Laboratories, Alexander Stone Building, Garscube Estate, Switchback Road, Bearsden, Glasgow G61; H.M. McCallum is at the Department of Pathology, Victoria Infirmary, Glasgow G42 9TY; and W.D. George is at the Glasgow University, Department of Surgery, Western Infirmary, Dumbarton Road, Glasgow G11, U.K.
Revised 25 Feb. 1993; accepted 9 Mar. 1993.

In view of our recent observation that the gene for topoisomerase II α and *erb*B2 are co-amplified in an adenocarcinoma cell line [7], we analysed breast cancer biopsy specimens for co-amplification of genes on chromosome 17q. We analysed 50 tumours and observed 6 cases with amplification of *erb*B2 of which 3 had co-amplification of *erb*B2, topoisomerase II α and RAR α genes. In addition, as part of our studies on genetic alterations at topoisomerase loci, we have used RFLP to examine LOH at topoisomerase loci and show allelic deletion at the topoisomerase I locus on chromosome 20 [13]. Allelic loss at the topoisomerase I locus may be of relevance to the aquisition of resistance to inhibitors of topoisomerase I activity.

MATERIALS AND METHODS

Breast tumour samples

All samples are primary untreated invasive ductal carcinomas except one which was an invasive lobular cancer. Tumours were obtained from co-operating hospitals in Glasgow and frozen at −70°C until use. All samples were collected under the routine hospital policy of consent. Clinical data on the 3 patients whose tumours show co-amplification of *erb*B2, RAR α and topoisomerase II α (Table 1) is as follows: Patient 3, 87-year-old, ductal carcinoma, grade III, node positive, oestrogen receptor negative. Patient 1, 47-year-old, ductal carcinoma, grade II, node positive, oestrogen receptor positive. Patient 2, 87-year-old, ductal carcinoma with papillary features and some mucin production, grade III, node negative, oestrogen receptor negative.

DNA extraction and Southern hybridisation

Genomic DNA was extracted from breast tumour tissue, normal breast tissue and white blood cells [7]. DNA was digested with restriction endonucleases according to manufacturer's directions, fractionated by agarose gel electrophoresis and transferred to nylon filters. Hybridisations were carried out as described previously [7]. DNA extracted from normal tissue was included on all blots. All blots were hybridised with multiple probes to assess gene copy number and allelic imbalance.

Sequential extraction of protein and DNA

We have developed methodology which allows the sequential extraction of protein and genomic DNA from the same biopsy sample to study topoisomerase II loci and their expression. This methodology permits data on gene expression to be correlated directly with genetic alterations. Frozen biopsy samples were pulverised under liquid nitrogen and protein extracted in 0.35 mol/l NaCl as described by van der Zee *et al.* [14]. The protein extract contains topoisomerase enzymes [14]. After extraction of proteins in 0.35 mol/l NaCl, insoluble material which includes the nuclei was pelleted and resuspended in 200 μl lysis buffer [0.3 mol/l NaCl, 5 mm EDTA, 0.5% sodium dodecyl sulphate (SDS)] and proteinase K added to 50 μg/ml. Lysis took place overnight at 37°C with mixing after which samples were extracted with phenol/chloroform and the DNA recovered by precipitation. Western blot analysis of the proteins was then carried out. For tumour samples 100 μg of protein was loaded per lane and 50 μg of MCF7 cell line protein extract. Samples were resolved by 6% SDS polyacylamide gel electrophoresis (PAGE) and proteins transferred to immobilon P (Millipore). Polyclonal antibody against topoisomerase II α was purchased from Cambridge Research Biologicals and antibody complexes visualised by chemiluminesence (Amersham).

DNA probes

The DNA probes used to characterise the topoisomerase loci are described in Table 2. The *erb*B2 probe is pCER204, PKC α probe is phPKC-alpha 7 [15], RAR α probe is p63, all of which were obtained from the ATCC. Probes for D20S4 [16] and SRC [17] were obtained through the UK Human Genome Mapping Project Resource Centre. PTP1B [18] was obtained from Dr David Hill.

RESULTS

Co-amplification of loci on chromosome 17q

DNA extracted from 50 breast tumours were analysed for amplification of genes on chromosome 17. Of the 50 samples, six had amplification of *erb*B2, three of which showed co-

Table 1. (*a*) *Frequency of co-amplification of topoisomerase II α, RAR α and* erbB2

	Genes amplified				
	*erb*B2	*erb*B2 only	Co-amplification *erb*B2, RAR α, TOPOII α	PKC α	Total number tumours
No of tumours with amplification	6	3	3	0	50

Table 1.(*b*) *Levels of gene amplification*

	Locus		
Tumour	*erb*B2	RAR α	TOPOII α
1	7	8	15
2	5	6	5
3	5	16	8

The three tumours showing co-amplification of *erb*B2, RAR α and topoisomerase II α (TOPOIIα) were analysed for levels of gene amplification. The numbers in Table 1(b) refer to the number of gene copies in the tumour in comparison to matched normal DNA.

Table 2.

Locus	Probe	Enzyme	Allele	Fragment length	Frequency	No. chrom analysed	Heterozygosity	Ref.
TOPOII α	ZII69	*Xmn* 1	A1	19	0.167	48	n.d.	20
			A2	7.2	0.833			
	hTOP2-Z2	*Xmn* 1	a1	19	0.14	184	22	This study and 7
			a2	8.5	0.86			
TOPOII β	SP12	*Pst* 1	A1	6	0.1	n.d.	14	19
			A2	5.5	0.9			
			A3	5	0.04			
			A4	3.5	0.96			
			b1	6	0.03	206	4	This study and 7
			b2	5.6	0.97			
			b3	5	0.16	206	24	
			b4	3.5	0.84			
TOPOI	pSCL 70	*Taq* 1	A1	8	0.32	136	n.d.	21
			A2	7	0.60			
			A3	5.4	0.07			
			A1	8	0.36	198	57	This study
			A2	7	0.61			
			A3	5.4	0.03			

Allele fragment sizes (kb), probes used and allele frequencies for characterisation of topoisomerase loci in normal tissue samples.

amplification of topoisomerase II α, RAR α and *erb*B2 genes (Table 1a). Figure 1 shows Southern blots of DNA extracted from tumour material and normal tissue from the two representative cases showing co-amplification of *erb*B2, topoisomerase II α and RAR α. All three genes have previously been localised to chromosome 17, region q 21-22 [8, 9, 11]. In order to control for loading of the gel, the filters were hybridised with an immunoglobulin heavy chain probe (pHJi) which maps to chromosome 14 [7]. Hybridisation of the filter with a probe for protein kinase C α (PKC α) which is localised to 17q 22-q24 [15] demonstrates that the over-representation of *erb*B2, topoisomerase II α and RAR α sequences is not due to aneuploidy as the intensity of their hybridisation follows that of pHJi. Although only representative parts of the autoradiographs are shown in Fig. 1, gross rearrangement of the amplified genes were not detected. Three tumours had amplification of *erb*B2 alone. Three tumours had co-amplification of topoisomerase II α, RAR α and *erb*B2 (Table 1a). Laser scanning densitrometry was used to quantitate levels of gene amplification in tumours compared to normal tissue. Table 1b shows that the topoisomerase II α gene is amplified by 5–15-fold in breast tumours.

Expression of topoisomerase II α

In order to assess whether topoisomerase II α was expressed in breast tumours, five samples were processed sequentially for protein extraction and DNA extraction as described in the Materials and Methods section. This enabled the genetic and expression analysis to be carried out from the same small biopsy specimen. As shown in Fig. 2, of the five tumour samples analysed in this fashion, two expressed topoisomerase II α protein. The topoisomerase II α protein detected in lane 5 of Fig. 2 shows some degradation, however, high levels of expression are detected in the tumour sample analysed in lane 6 of Fig. 2. The sample shown in lane 6 (Fig. 2) is protein extracted from tumour sample 2 shown in Fig. 1 which has an amplified topoisomerase II α gene. None of the other samples shown in Fig. 2 have amplified topoisomerase II α sequences thus, this approach is useful for combining genetic and expression analysis.

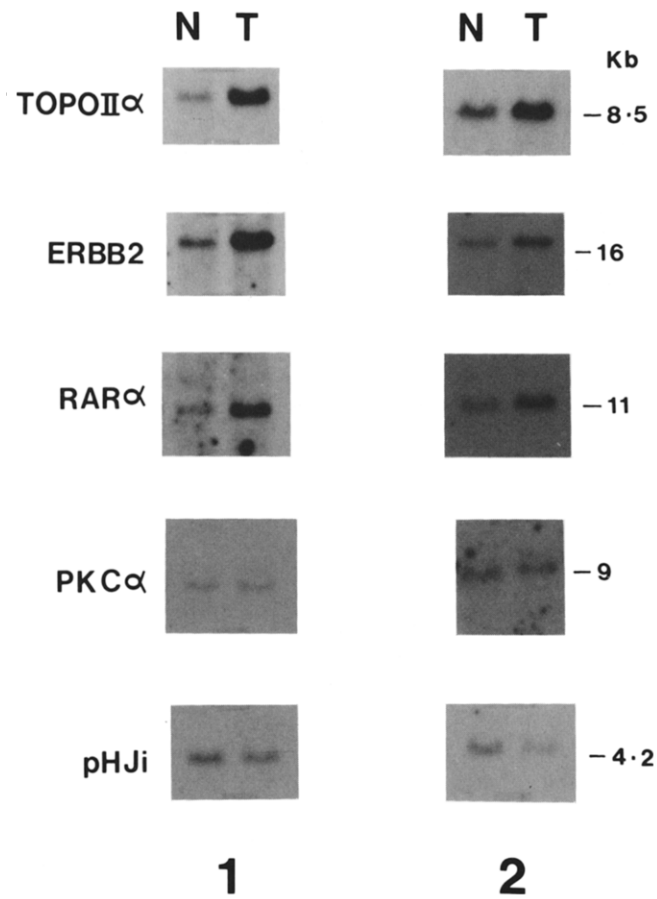

Fig. 1. Southern blot analysis of loci amplified in breast tumours. DNA extracted from normal tissue (N) and tumour biopsies (T) was digested with *Xmn* 1 and analysed by Southern blotting. Two paired samples, 1 and 2, are shown. Filters were sequentialy hybridised to the probes shown on the left. Fragment sizes are shown on the right.

Allele loss at topoisomerase loci

Topoisomerase II α, II β and topoisomerase I have been localised to chromosomes 17q, 3p and 20q, respectively [9, 13, 19] and RFLP have been described for all three loci [7, 19, 20, 21]. Table 2 summarises allele sizes, probes used and allele frequencies for characterisation of the three topoisomerase loci in normal tissue.

LOH at topoisomerase loci in breast cancer biopsy specimens is summarised in Table 3. From Table 3 it can been seen that 2 out of 9 informative individuals showed LOH at the topoisomerase II α locus on chromosome 17q. Figure 3a shows that in both samples 21 and 25, allele a1 has a diminished hybridisation signal when compared to allele a2 and the normal DNA samples. Hybridisation to residual copies of allele a1 is contaminating normal tissue or tumour heterogeneity and is exaggerated due to the unequal loading of normal and tumour DNA. Due to the limited amounts of DNA obtained from samples 21 and 25 no further analysis on the extent of allelic loss at other loci could be determined.

From Table 3 it can be seen that allelic loss at the topoisomerase I locus on chromosome 20q 11.2–13.2 occurred in 2 out of 17 informative samples. Figure 3b illustrates LOH at the topisomerase I locus in samples 24 and 27 as indicated by a diminished hybridisation signal in alleles A2 and A3, respectively. Hybridisation of the filter with pHJi to control for DNA loading shows that the allele imbalance is due to loss of heterozygosity.

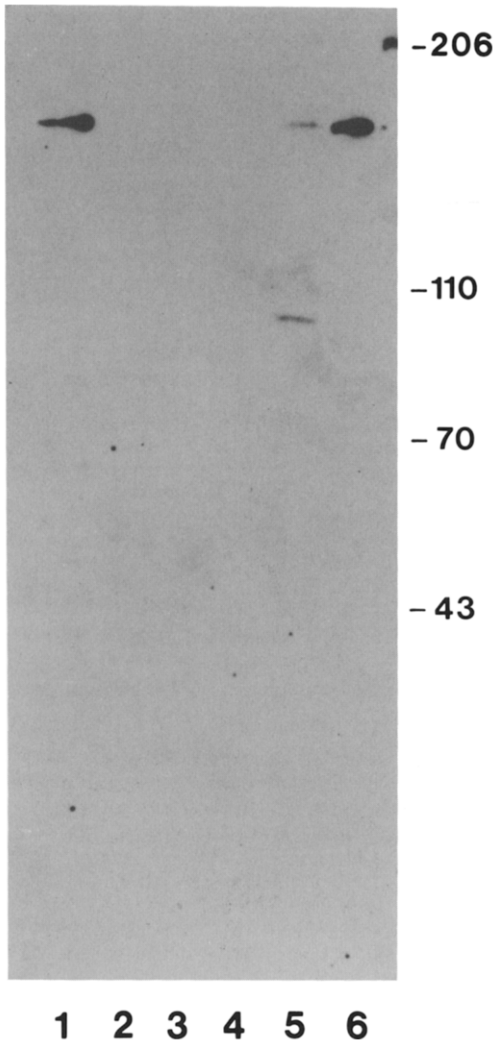

Fig. 2. Expression of topoisomerase II α in breast tumours. Proteins extracted from breast tumours (lanes 2–6) and from the breast cancer cell line MCF-7 (lane 1) were analysed by western blot for expression of topoisomerase II α. A protein of 170 kD was detected. Size markers are shown to the right of the figure and are in kD.

Table 3. LOH at topoisomerase loci in breast cancer

Locus	No.	HZ	LOH
TopoII α	31	9	2
TopoII β	21	4	0
TopoI	26	17	2

DNA extracted from paired samples of normal tissue and tumour from the same patient were analysed for loss of heterozygosity (LOH). The enzymes and probes used are described in Table 1. No., is the number of pairs analysed. Hz, is the number of heterozygotes. LOH is the number of heterozygotes showing allelic loss at each locus. Topo, topoisomerase.

Sufficient DNA was obtained from samples 24 and 27 to analyse other loci on chromosome 20 in an attempt to define the region lost. DNA was digested with *Msp*1 and transferred to a filter. D20S4 is a polymorphic marker on 20q 13.2 observed with *Msp*1 [16]. As can be seen from Fig. 3(c) sample 24 is heterozygous for alleles A1 and A2. The increased intensity of hybridisation to alleles A2 in the tumour in comparison to normal tissue is due to an increase in copy number of the locus D20S4 as shown by hybridisation of the filter with the control immunoglobulin probe pHJi rather than allele loss at D20S4 and an imbalance in DNA loading. Thus, sample 24 has LOH at the topoisomerase I locus and an increase in allele number at D20S4, suggesting D20S4 is outside the deleted region and that there has been reduplication of chromosome 20. Hybridisation of the filter shown in Fig. 3(c) to topoisomerase I confirms that although LOH at topoisomerase I has occurred (Fig. 3b), the remaining allele has reduplicated as the hybridisation intensity with topoisomerase I is similar in both the normal and tumour samples and similar to the intensity of hybridisation to the control probe pHJi. Therefore on reprobing the filter shown in Fig. 3(c), any chromosome 20 sequences which lie within the deletion will show a hybridisation intensity similar to pHJi or topoisomerase I. Sequences within the region of reduplication will show an intensity of hybridisation similar to D20S4. Subsequent hybridisation of the filter shown in Fig. 3(c) to chromosome 20q genes, SRC [17] and phosphotyrosyl phosphatase 1B (PTP1B) [18] demonstrates that the tyrosine kinase oncogene, SRC, lies within the region of loss whereas PTP1B is outside the deleted region.

Although sample 27 was not informative at D20S4 (Fig. 3c), from the data shown in Fig. 3(b, c) there appears to be allelic loss of all the markers examined with re-duplication of the remaining allele.

DISCUSSION

In the present study, we have analysed breast cancer biopsy specimens for genetic alterations at and around topoisomerase

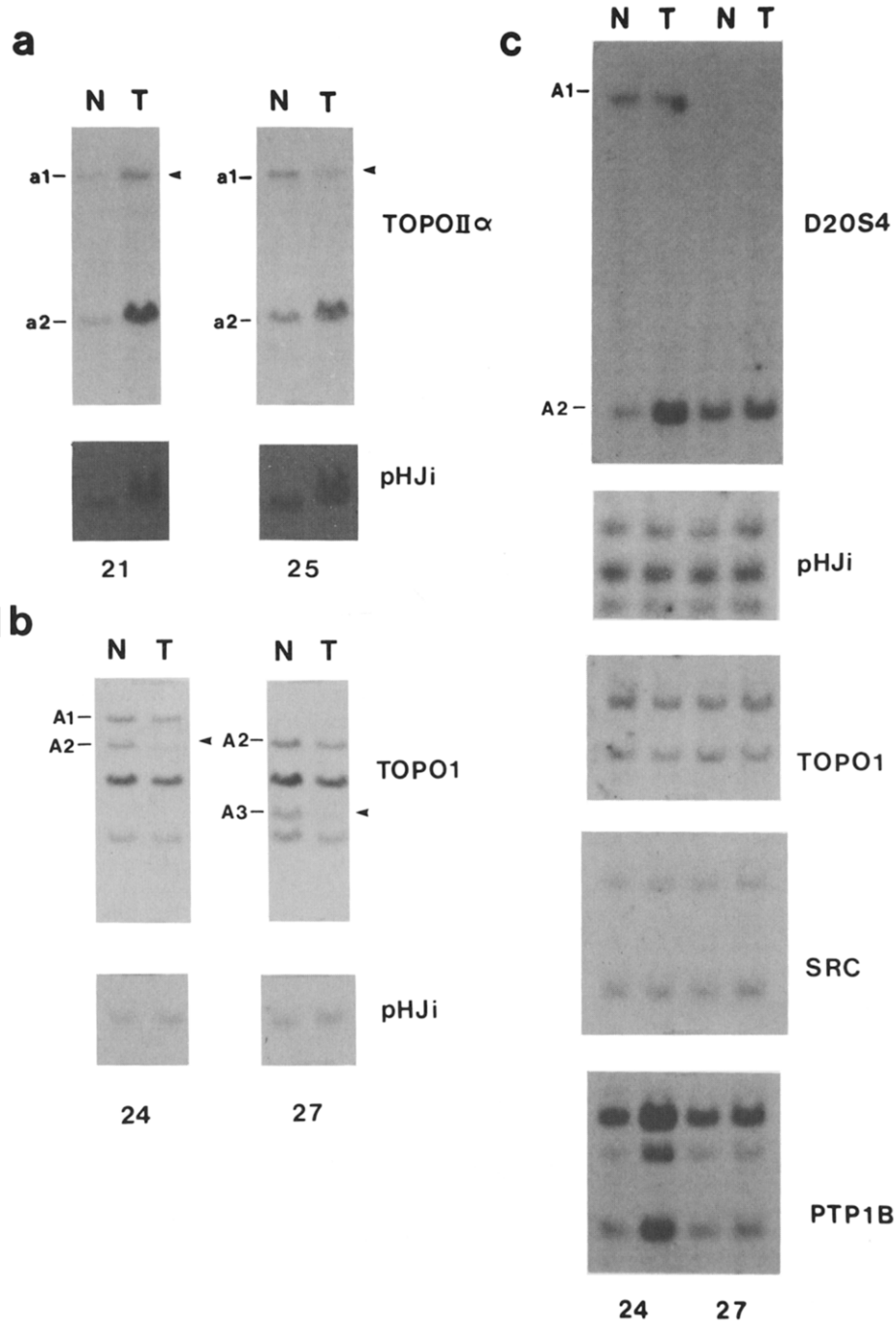

Fig. 3. RFLP analysis of topoisomerase genes. Southern blot analysis was carried out following restriction endonuclease digestion with the appropriate enzymes. Details of enzymes and topoisomerase probes are shown in Table 2. DNA was extracted from normal tissue (N) and tumour (T) for each sample. The sample number is shown below each autoradiograph. The gene detected on each autoradiograph is shown to the right. (a) RFLP analysis of topoisomerase II α gene. The two allelic fragments a1 and a2 are shown to the left of the autoradiograph and allelic loss is shown by an arrow head on the right. (b) RFLP analysis of the topoisomerase I gene. The three allelic fragments A1, A2, A3 are shown to the left of the autoradiograph and allelic loss is shown by arrow heads to the right. (c) Allelic imbalance on chromosome 20. DNA was digested with *Msp* 1 transferred to a filter which was sequentially probed with the genes shown to the right of the autoradiographs. The probe D20S4 detects the alleles A1 and A2 and are shown to the left of the autoradiograph. Sample 24 is heterozygous at D20S4 and the tumour (T) shows allelic imbalance. All filters were probed with the immunoglobulin probe pHJi to control for loading.

loci. The molecular analysis of breast tumours carried out in this study identifies for the first time co-amplification of the RAR α and topoisomerase II α genes in primary biopsy samples. Both RAR α and topoisomerase II α have been mapped to chromosome 17q 21 [9, 11] and have undergone co-amplification due to their proximity to the *erb*B2 oncogene [7].

The data presented in Table 1 and Fig. 1 show that out of six tumours with amplification of *erb*B2, three show co-amplification of topoisomerase II α, RAR α and *erb*B2 genes. Topoisomerase II is involved in a number of basic cellular functions and deregulated expression may affect chromosome segregation and recombination [5]. Thus, further studies on topoisomerases will

reveal whether an altered topoisomerase will have a phenotypic effect in terms of tumour development. To return to factors which may influence a tumour response to anticancer agents, the topoisomerase inhibitors are an exciting class of drugs which are quite commonly used clinically. High levels of topoisomerase II α enzyme would present a target for topoisomerase inhibitory drugs such as VP16 or doxorubicin and tumours expressing increased levels would be sensitive to the cytotoxic effects of these agents. By sequential extraction of protein and DNA from a number of breast tumours (Fig. 2) it can be shown that a tumour with an amplified topoisomerase II α gene (Fig. 1, sample 2) also expresses topoisomerase II α protein (Fig. 2, lane 6). These data suggest that at least a sub-population of breast cancers may benefit from treatment with inhibitors of topoisomerase II due to fortuitous molecular changes during adenocarcinoma carcinogenesis. The recent finding that an adenocarcinoma cell line has co-amplification of topoisomerase II α and *erb*B2 [7] should provide a useful cell line model in which to study the regulation of expression of an amplified topoisomerase II α gene.

The RAR α receptor is of interest both as a possible participant in tumorogenesis and as mediator of the anticancer effects of retinoids [10]. Acute promyelocytic leukaemia (APL) is hallmarked by a balanced translocation between chromosomes 17q and 15q which fuses the RAR α gene with a gene coding for a putative DNA binding protein named PML [10, 12]. The PML-RAR α fusion protein exhibits altered transcriptional transactivating properties and many act as an oncogene as a result of this. The PML-RAR α fusion protein is, however, still hormone responsive and patients with APL respond well to treatment with retinoids [12]. Our observation (Table 1, Fig. 1) that in 3 cases of breast cancer, the RAR α gene is amplified is of interest due to its possible participation in oncogenesis [12] and as a mediator of the cytostatic effects of retinoid treatment [10]. The data presented on amplification of the RAR α receptor (Table 1, Fig. 1), may indicate a subgroup of breast cancer patients who would benefit from treatment with retinoids.

The co-amplification of topoisomerase II α, RAR α and *erb*B2 in three independent tumours suggests physical linkage on chromosome 17q. The exact order of these genes on 17q is at present undefined. However, the present data may aid the description of the physical map of this region important in both sporadic and familial early onset breast cancer [22, 23]. The amplification of *erb*B2 remains the most likely molecular event which is selected for during adenocarcinoma carcinogenesis as in no case was amplification of RAR α or topoisomerase II α seen without *erb*B2, whereas in 3 cases amplification of *erb*B2 was seen alone (Table 1a). However, the phenotypic effects of overexpression of RAR α and topoisomerase II α during tumour development requires more detailed analysis as it may play a role in carcinogenesis after the amplification of *erb*B2. Amplification of genes linked to *erb*B2 may provide molecular marker with which to establish new prognostic markers and sub-divide a group of tumours with amplified *erb*B2 which generally have a poor prognosis [3]. The data presented a co-amplification and expression of topoisomerase II α and co-amplification of RAR α may prove useful in treatment design and refining prognostic markers.

Somatic loss of loci on chromosomes 17 and 3 is relatively well documented in breast cancer [1] and the data presented on topoisomerase II α and β provide further genetic markers for investigation (Tables 2 and 3, Fig. 3a). However, we also describe loss of heterozygosity at the topoisomerase I locus on chromosome 20q in two out of 17 heterozygotes (Table 3, Fig. 3b). Deletion of chromosome 20q sequences is a hallmark of a number of haematological diseases [24–26] suggesting the presence of tumour suppressor genes in this region. A recent report by Devillee *et al.* [23] demonstrated allelic imbalance on chromosome 20q at a frequency of 18% in breast cancer. It is, therefore, a possibility that there are genes located on chromosome 20q whose alteration may be causative in a population of breast tumours. The data presented in Table 3 and Fig. 3 on allelic imbalances of genes localised to chromosome 20 add weight to this region being important in breast cancer development. In the two cases of breast cancer which showed LOH at the topoisomerase I locus another polymorphic marker on chromosome 20q was examined, namely D20S4 [16]. Sample 24 showed a gain in allele A2 at D20S4. Thus, sample 24 has undergone LOH at the topoisomerase I locus followed by reduplication of the remaining allele to give the allelic gain at D20S4. The data presented in Fig. 3(c) for tumour sample 24 suggests that the putative tumour suppressor gene PTP 1B [18] is beyond the deletion on chromosome 20q. Interestingly, the SRC tyrosine kinase oncogene is within the deleted region. Allele loss of SRC has been demonstrated in two cases of leukaemias with cytogenetic 20q deletions [24] and so this region of chromosome 20 may be important in tumour development. The data presented an allele loss on chromosome 20 including topoisomerase I and SRC suggest these genes may be linked as 20q. However, only an extended study on allele loss in this region will determine the causative events for this loss. It is, of course, a possibility that these losses may be random due to genome instability in the tumour. Loss of heterozygosity is one mechanism by which recessive mutations at loci can become expressed. Mutations in topoisomerase genes have been identified in cell lines selected for resistance to topoisomerase inhibitors [27–29]. It, therefore, remains a possibility that should LOH at topoisomerase loci occur during or prior to chemotherapy, as a result of linked oncogenic changes, mutation in the remaining allele may give rise to a population of cells resistant to the topoisomerase inhibitors. Of particular relevance is an mAMSA resistant subline of HL-60 which shows LOH at the topoisomerase II α locus during selection for resistance as well as mutation of the gene [20, 29, 30]. Tamura *et al.* [27] have identified a mutation in a topoisomerase I gene which confers resistance to camptothecin. Camptothecin analogues are useful topoisomerase I inhibitors and are used clinically. Our data on LOH at the topoisomerase I locus is one mechanism by which a mutation could arise and contribute to drug resistance. The characterisation of restriction fragment length polymorphisms at topoisomerase loci (Table 2) and their use in defining allele imbalances in tumour biopsy specimens are important as the clinical use of topoisomerase inhibitors increases.

In conclusion, we have shown in breast tumours co-amplification of RAR α, *erb*B2 and topoisomerase II α genes. Although the contributions of RAR α and topoisomerase II α amplifications to breast tumour progression are unknown, the expression of RAR α and topoisomerase II α generates targets for anticancer drug treatment. The high level of topoisomerase II α protein described in tumour sample number 2 (Fig. 2), which has an amplified topoisomerase II α gene, demonstrates a molecular basis for drug treatment. Alterations at topoisomerase loci appear to be relatively frequent in breast cancer. It is unlikely, however, that these are primary events in tumour development and amplification of topoisomerase II α is a result of linkage to *erb*B2. As to the causative genetic changes on chromosome 20, there is little data on allele imbalances at the

genes situated there. The data presented here and by others suggests a region encompassing topoisomerase I and SRC may be of importance in carcinogenesis.

1. Deville P, Cornelisse CJ. Genetics of human breast cancer. *Cancer Surveys* 1990, **9**, 605–630.
2. Gullick WJ. The role of the epidermal growth factor receptor and the c-*erb*B2 protein in breast cancer. *Int J Cancer* 1990, 5, 55–61, (Suppl).
3. Perren TJ. c-*erb*B-2 Oncogene as a prognostic marker in breast cancer. *Br J Cancer* 1991, **63**, 328–332.
4. Liu LF. DNA topoisomerase poisons as antitumour drugs. *Ann Rev Biochem 1989*, **58**, 351–375.
5. Wang JC, Caron PR, Kim RA. The role of DNA topoisomerases in recombination and genome stability: a double-edged sword? *Cell* 1990, **62**, 403–406.
6. Takano H, Kohno K, Matsuo K, Matsuda T, Kuwano M. DNA topoisomerase targeting antitumour agents and drug resistance. *Anti-Cancer Drugs* 1992, **3**, 323–330.
7. Keith WN, Tan KB, Brown R. Amplification of the topoisomerase II α gene in a non-small cell lung cancer cell line and characterisation of polymorphisms at the human topoisomerase II α and β loci in normal tissue. *Genes Chromosomes Cancer* 1992, **4**, 169–175.
8. Fukushige SI, Matsubara KI, Yoshida M, *et al.* Localisation of a novel v-*erb*B related gene, c-*erb*B-2, on human chromosome 17 and its amplification in a gastric cancer cell line. *Mol Cell Biol* 1986, **6**, 955–958.
9. Tsai-Pflugfelder M, Liu LF, Liu AA, *et al.* Cloning and sequencing of cDNA encoding human DNA topoisomerase II and localisation of the gene to chromosome regions 17q 21-22. *Proc Natl Acad Sci USA* 1988, **85**, 7177–7181.
10. Bollag W, Holdener EE. Retinoids in cancer prevention and therapy. *Ann Oncol* 1992, **3**, 513–526.
11. Mattei MG, Petkovich M, Mattei JF, Brand N, Chambon P. Mapping of the human retinoic acid receptor to the q21 band of chromosomes 17. *Human Genet* 1988, **80**, 186–188.
12. Clarkson B. Retinoic acide in acute promyelocytic leukaemia: the promise and the paradox. *Cancer Cells* 1991, **3**, 211–220.
13. Kunze N, Yang GC, Jiang ZY *et al.* Localisation of the active type 1 DNA topoisomerase gene on human chromosome 20q 11.2-13.1, and two pseudogenes on chromosomes 1q 23-24 and 22q 11.2-13.1. *Human Genet* 1989, **84**, 6–10.
14. Van der Zee AGJ, Hollema H, de Jong S, *et al.* P-Glycoprotein expression and DNA topoisomerase I and II activity in benign tumours of the ovary and malignant tumours of the ovary, before and after platinum/cyclophosphamide chemotherapy. *Cancer Res* 1991, **51**, 5915–5920.
15. Parker PJ, Coussens L, Totty N, *et al.* The complete primary structure of protein C the major phorbol ester receptor. *Science* 1986, **233**, 853–859.
16. Goodfellow PJ, Duncan AMV, Farrar LA, *et al.* Localisation and linkage of three polymorphic DNA sequences in human chromosome 20. *Cytogenet Cell Genet* 1987, **44**, 112–117.
17. Parker RC, Mardon G, Lebo RV, Varmus HE, Bishop JM. Isolation of duplicated human c-*src* genes located on chromosomes 1 and 20. *Mol Cell Biol* 1985, **5**, 831–838.
18. Brown-Shimer S, Johnson KA, Lawerence JB, *et al.* Molecular cloning and chromosome mapping of the human gene encoding protein phosphotyrosyl phosphotase 1B. *Proc Natl Acad Sci USA* 1990, **87**, 5148–5152.
19. Tan KB, Dorman TE, Falls KM, *et al.* Topoisomerase II α and topoisomerase II β genes: characterisation and mapping to human chromosomes 17 and 3 respectively. *Cancer Res* 1992, **52**, 231–234.
20. Zwelling LA, Mayes J, Deisseroth K, *et al.* A restriction fragment length polymorphism for human topoisomerase II: possible relationships to drug resistance. *Cancer Comm* 1990, **2**, 357–361.
21. Sunde L, Kjeldsen E, Andoh T, Keene D, Bolund L. A three allele TaqI polymorphism at TOP1 gene. *Nucleic Acid Res* 1990, **18**, 5919.
22. Fain PR. Third international workshop on human chromosome 17 mapping. *Cytogenet Cell Genet* 1992, **60**, 178–186.
23. Devilee P, van Vliet M, van Sloun, Kuipers Dijkshoorn N, Hermans J, Pearson PL, Cornelisse CJ. Allelotype of human breast carcinoma: a second major site for loss of heterozygosity is on chromosome 6q. *Oncogene* 1991, **6**, 1705–1711.
24. Morris CM, Honeybone LM, Hollings PE, Fitzgerald PH. Localisation of the SRC oncogene to chromosome band 20q 11.2 and loss of this gene with deletion (20q) in two leukemic patients. *Blood* 1989, **74**, 1768–1773.
25. Le Beau M, Westbrook CA, Diaz MO, Rowley J. c-*src* is consistently conserved in the chromosomal deletion (20q) observed in myeloid disorders. *Proc Natl Acad Sci USA* 1985, **82**, 6692–6696.
26. Davis MP, Dewald GW, Pierre RV, Hoagland HC. Hematological manifestations associated with deletions of the long arm of chromosome 20. *Cancer Genet Cytogenet* 1984, **12**, 63–71.
27. Tamura H, Kohchi C, Yamada R, *et al.* Molecular cloning of a cDNA of a camptothecin-resistant human DNA topoisomerase 1 and identification of mutation sites. *Nucleic Acids Res* 1991, **19**, 69–75.
28. Bugg BY, Danks MK, Beck WT, Suttle DP. Expression of a mutant DNA topoisomerase II in CCRF-CEM human leukemic cells selected for a resistance to teniposide. *Proc Natl Acad Sci USA* 1991, **88**, 7654–7658.
29. Hinds M, Deisseroth K, Mayes J, *et al.* Identification of a point mutation in the topoisomerase II gene from a human leukemia cell line containing an amsacrine-resistant form of topoisomerase II. *Cancer Res* 1991, **51**, 4729–4731.
30. Zwelling LA, Hinds M, Chan D, *et al.* Characterisation of an amsacrine - resistant line of human leukemia cells. Evidence for a drug-resistant form of topoisomerase II. *J Biol Chem* 1989, **264**, 16411–16420.

Acknowledgements—The authors would like to thank Dr D. Hill, Dr Liu, Dr K.B. Tan and Dr E. Kjeldsen for providing the probes used in this study. The authors would also like to thank Professor P. Workman and J. Rose for valuable and stimulating discussion. This work was supported by the Cancer Research Campaign of Great Britain.