Eur J Cancer, Vol. 26, No. 4, pp. 423–429, 1990.
Printed in Great Britain

0277-5379/90$3.00 + 0.00

Papers

Gene Amplification on Chromosome Band 11q13 and Oestrogen Receptor Status in Breast Cancer

V. Fantl, M.A. Richards, R. Smith, G.A. Lammie, G. Johnstone, D. Allen, W. Gregory, G. Peters, C. Dickson and D.M. Barnes

We have analysed DNA from 183 primary breast cancers for amplification or rearrangement of a number of cellular proto-oncogenes, focusing primarily on a cluster of markers on the long arm of chromosome 11. Two of these oncogenes, *INT2* and *HST1*, both of which encode members of the fibroblast growth factor family, are implicated in the generation of virally induced mammary tumours in mice. Here we confirm earlier reports that the q13 region of chromosome 11, in which these genes are tandemly linked, is modestly amplified in approximately 15% of primary human breast cancers. This amplification is confined, with one exception, to cases in which the oestrogen receptor (ER) levels are in excess of 20 fmol/mg protein ($P = 0.001$). However, DNA amplification does not usually result in detectable expression of either the *INT2* or *HST1* gene. The data imply that some other gene in the vicinity must contribute to the development of a subset of ER-positive tumours and that assessing the amplification of this region of DNA may be of value in defining a separate category of ER-positive tumour.
Eur J Cancer, Vol. 26, No. 4, pp. 423–429, 1990.

INTRODUCTION

THE IDENTIFICATION of specific cellular genes whose properties or activities are perturbed by chromosomal aberrations in tumour cells holds considerable promise for the diagnosis and treatment of human cancer. Many of the recognized oncogenes function normally in controlling the growth and differentiation of specific cell types and contribute to malignancy only as a result of inappropriate expression, for example, in the wrong cell type, at the wrong time or at the wrong level, or through structural alterations that affect the properties of the encoded product (reviewed in [1]). The molecular events that can unmask the latent properties of oncogenes range from simple point mutations to major chromosomal abnormalities, including amplification, deletion or translocation of specific segments of DNA [1]. Thus, although many of the known oncogenes were originally identified as targets for oncogenic viruses in animal models, it is now clear that the same or similar genes may feature in particular human tumours with no known viral aetiology.

For these reasons, we and others have been investigating the possible involvement of the human *INT2* and *HST1* genes in primary breast cancer. Both these genes have been implicated in virally induced mammary tumours in mice, where their expression is induced by the nearby insertion of mouse mammary tumour virus DNA [2–4]. Both encode proteins that belong to the fibroblast growth factor family [2, 5–7] and they are closely linked on mouse chromosome 7 and on band q13 of human chromosome 11 [3, 8–11]. Several independent studies have shown that this latter segment of DNA is amplified in about 10–20% of human breast cancers, as well as in some other malignancies [8, 11–18]. However, it is not clear that either of the two genes plays a significant role in the development of the tumour since the region of amplified DNA is large enough to include several other markers [17, 19, 20]. Moreover, although there is a tentative correlation with early recurrence, the prognostic significance of the amplification has yet to be firmly established [12, 19, 21, 22]. Here we report the current status of our analysis of 183 primary tumours from patients who presented to the ICRF Clinical Oncology Unit at Guy's Hospital between 1986 and 1988, and show that although the *INT2* and *HST1* genes are unlikely to be important in tumour progression, the presence of the amplified unit may distinguish a separate category among ER-positive tumours.

Correspondence to G. Peters.
V. Fantl, R. Smith, D. Allen and C. Dickson are at the Imperial Cancer Research Fund Laboratories, Lincoln's Inn Fields, London WC2A 3PX, U.K., M.A. Richards, G. Johnstone, W. Gregory and D.M. Barnes are at the ICRF Clinical Oncology Unit, Guy's Hospital, London SE1 9RT, U.K. and G.A. Lammie and G. Peters are at the ICRF Laboratories, St Batholomew's Hospital, Dominion House, Bartholomew Close, London EC1A 7BE, U.K.

PATIENTS AND METHODS

Clinicopathological characteristics of the study population

The present study was conducted with primary tumour tissue obtained from 183 patients who presented with infiltrating breast carcinoma to the Guy's Hospital Clinical Oncology Unit between 1986 and 1988. The clinicopathological characteristics of these patients are summarized in Table 1. The median age was 58 years (range 27–84 years). Patients who had menstruated within a year of presentation were considered premenopausal (27%). Nine patients (5%) presented with overt metastatic spread of cancer, while a further 32 (17%) had locally advanced, inoperable tumours. Of the 142 patients (78%) who presented with operable disease, 121 underwent either a modified radical mastectomy or a conservation procedure involving tumourectomy, axillary clearance and radiotherapy. The remaining 21 patients, all aged over 70 years, underwent wide excision of the primary tumour, but did not have an axillary clearance.

Tumour size was measured clinically. Oestrogen receptor (ER) and progesterone receptor (PR) levels were measured on 173 of the 183 tumours by enzyme immunoassay (ER-EIA and PR-EIA kits, Abbott). Receptor levels of ≥ 20 fmol/mg of cytosolic protein were considered positive. Histological type was recorded for all tumours, ductal carcinomas being graded according to the method of Bloom and Richardson [23].

Southern blot analyses of tumour DNA

As described in a previous report [17], total cellular DNA was isolated from primary breast tumour tissue using an Applied Biosystems 340A DNA extractor. Between 0.3 and 0.5 g of tissue was frozen in liquid nitrogen and disaggregated using a Braun Mikro-dismembrator II prior to extraction. Samples (15 μg) were digested with the restriction enzyme *Pst*I and fractionated by electrophoresis in 0.8% agarose gels. Gels were treated with 0.25 M HCl for 10 min and the DNA was transferred to Genescreen Plus membranes by blotting in 0.4 M NaOH, essentially as described by Reed and Mann [24]. Filters were hybridized simultaneously with ^{32}P-labeled probes for several different markers (see below) prepared by priming purified restriction fragments with random oligonucleotides (Amersham International plc). The conditions used for hybridization and washing were based on those described by Church and Gilbert [25].

Specifications of probes

The probes used in these analyses were (i) a 0.4 kb *Bam*HI-*Sac*I fragment from human *INT2*, derived from the 1.0 kb fragment designated BK4 [9, 26]; (ii) the 0.79 kb *Eco*RI fragment from human *HST1* designated fragment c by Sakamoto *et al.* [27]; (iii) a 0.6 kb *Sac*I-*Hind*III fragment from human *BCL1* derived from the *Sac*I fragment originally designated probe b by Tsujimoto *et al.* [28]; and (iv) a 0.78 kb *Eco*RI fragment from the *CD3*γ-chain cDNA clone pJ6T3γ-2[29].

RNase protection assays

Total RNA was extracted from frozen, dismembranated tissues by homogenization in guanidinium thiocyanate and centrifugation through caesium chloride as described elsewhere [30, 31]. Samples (20 μg) of total RNA were used directly in RNase protection assays with an antisense probe corresponding to nucleotides 9232–9620 of the human *INT2* sequence described by Brookes *et al.* [26]. As a control for RNA loading and integrity, an antisense probe from the human γ-actin gene was included in each sample.

Table 1. *Patient characteristics*

		(n = 183)
Age		
≤ 50		52
51–70		92
> 70		39
Menstrual status		
Premenopausal		53
Postmenopausal		130
Extent of disease		
I/II operable:	node negative	47
	node positive	74
	nodes not examined	21
III	locally advanced	32
IV	metastatic	9
Tumour size		
≤ 2 cm		27
2.1–5.0 cm		114
> 5 cm		42
Histology		
Ductal grades I/II		72
Ductal grade III		63
Lobular		25
Other		23
Oestrogen receptor		
< fmol/mg		55
≥ 20 fmol/mg		118
ND		10
Progesterone receptor		
< 20 fmol/mg		83
≥ 20 fmol/mg		89
ND		11

RESULTS

Analysis of tumour DNAs

DNA from each of 183 breast cancers was subjected to Southern blot analysis using probes corresponding to the *BCL1*, *INT2* and *HST1* loci on chromosome 11q13, and the *CD3*γ-chain. The latter maps more distally (band q23) on the long arm of chromosome 11 [32] and served as a control for chromosome loss or duplication, as well as an internal control for DNA loading. The degree of amplification of the 11q13 markers was assessed relative to *CD3*γ, and generally ranged between 2- and 8-fold. An example of such an analysis is shown in Fig. 1. In this experiment, tumour DNA was compared to matched peripheral blood lymphocyte DNA as a further control for the presence of the amplified segment. A total of 28 of the 183 tumours analysed (15%) showed significant amplification of *INT2*, and in 26 of these cases, all three 11q13 markers were co-amplified to approximately equivalent degrees. The two exceptions were tumours in which the *BCL1* locus was amplified to a lesser extent or not at all (summarized in Table 2).

This latter finding suggests that the *BCL1* locus cannot be the key feature in the amplified unit. However, the *BCL1* probe used here does not correspond to an expressed gene, but simply defines a region involved in reciprocal chromosomal translocations between 11q13 and the immunoglobulin heavy

Table 2. Co-amplification of 11q13 markers

Locus	Total tumours analysed	Tumours with amplification	Degree of amplification (copy number) ~2	2–4	4–6	> 6
INT2	183	28	3	14	8	3
HST1	183	28	3	14	8	3
BCL1	183	27	4	12	8	3

chain locus on 14q32 in certain lymphoid malignancies [28]. As the affected gene may be a considerable distance from the breakpoint, it is not yet excluded as a contributing factor in breast cancer. We have also assessed other markers from the region, such as the *pMS51* minisatellite probe [14] and the *SEA* oncogene [33], but only in a subset of the tumours. While it is often included in the amplified unit, in the 50 cases analysed, *pMS51* was less consistently amplified than *INT2* and *HST1*. In contrast, we detected no abnormalities in the *SEA* gene in several tumours where *INT2* was clearly amplified.

Analysis of tumour RNAs

A critical test of whether a gene contributes to the development of disease is whether it is expressed in the affected tissue, as judged by detection of either messenger RNA or protein. It was therefore important to analyse the tumours for evidence of *INT2* and *HST1* expression. Experience with the murine homologues suggested that levels of expression could be low and might preclude immunological detection of the products [3, 31]. Moreover, preliminary attempts to detect *INT2*-specific RNAs by blot hybridization proved negative. To improve the sensitivity,

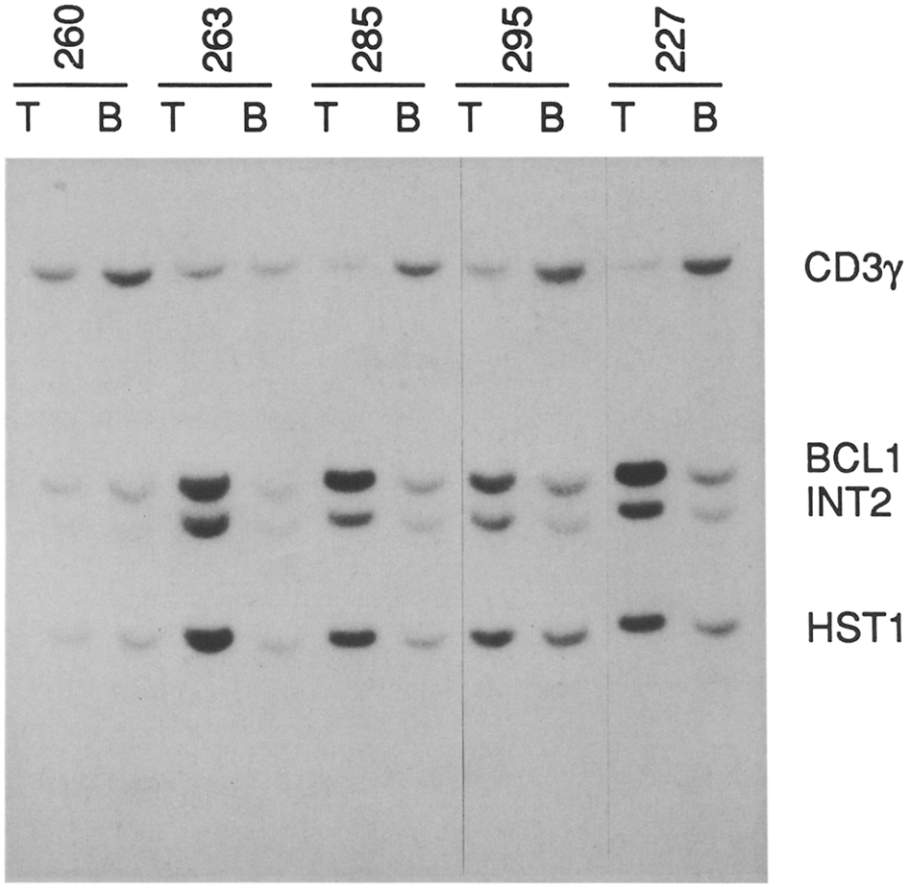

Fig. 1. Southern blot analysis of breast tumour DNAs. Samples (15 μg) of matched tumour (T) and blood lymphocyte (B) DNA were digested with *PstI* and fractionated by electrophoresis in a 0.8% agarose gel. After transfer to a nylon membrane, the DNA samples were hybridized simultaneously to ^{32}P-labelled probes for the *CD3γ*, *BCL1*, *INT2* and *HST1* loci. Hybridizing bands were detected by autoradiography. Tumours 263, 285, 295 and 227 are representative cases in which all three markers in the 11q13 region are co-amplified, whereas 260 shows no amplification at these loci.

we elected to use the ribonuclease (RNase) protection assay which is capable of detecting as little as one RNA molecule per cell. This technique had previously enabled us to analyse the 1.7 kb *INT2* transcript seen in the human teratocarcinoma cell line, Tera-2, and to confirm the structure of the three coding exons [26]. In the experiment illustrated in Fig. 2, the antisense RNA probe included 386 nucleotides from exon III such that an RNase-resistant duplex of this length was evident with Tera-2 cell RNA but not with the control tRNA (Fig. 2).

When similar assays were performed on equivalent amounts of RNA from selected mammary tumours, all but one of 28 tumours analysed scored negative for *INT2* expression, irrespective of any amplification of the gene. Figure 2 shows the results obtained for 12 of these tumours of which 5 (numbers 330, 144, 377, 123 and 108) had shown *INT2* DNA amplification. We also analysed RNA from normal mammary tissue, obtained through reduction mammoplasty (RM). The 386 base pair fragment characteristic of *INT2* RNA was visible in only one sample, tumour 123. This tumour was also exceptional in showing a DNA amplification that did not encompass *BCL1*. As a control for the amount and integrity of the tumour RNA, an antisense probe for the γ-actin gene was included in each assay, generating an RNase-resistant duplex of 130 base pairs. The signals from this abundant transcript were essentially

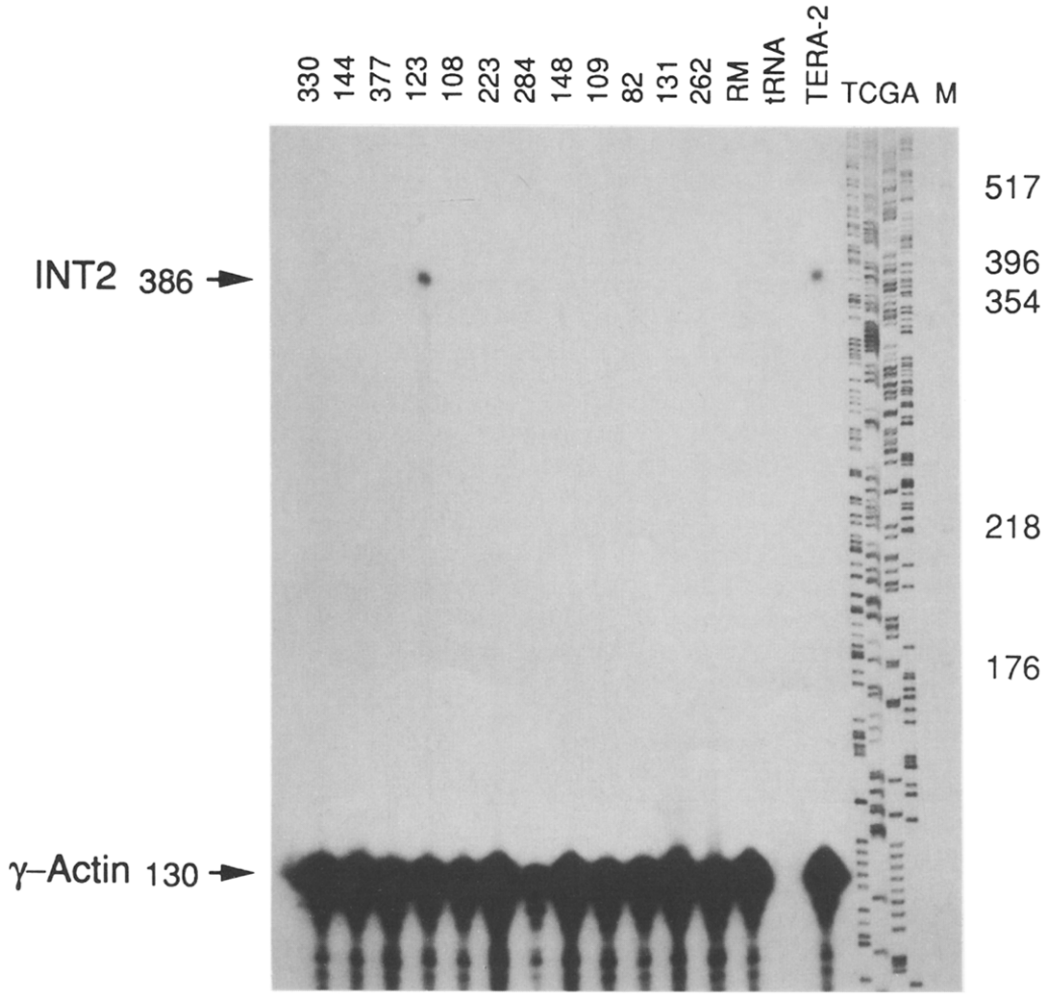

Fig. 2. RNase protection analysis of breast tumour RNA. Total cellular RNA (20 μg) from each of the numbered tumours, and a reduction mammoplasty (RM), was hybridized directly to ^{32}P-labelled antisense RNA probes specific for the human *INT2* and γ-actin genes. Non-hybridizing RNA was removed by digestion with RNases A and T1, and resistant duplexes were analysed by electrophoresis in a denaturing polyacrylamide gel [26]. The protected 386 nucleotide fragment was derived from the *INT2* transcript, while the γ-actin RNA yielded a characteristic protected fragment of 130 nucleotides. Total RNA from the Tera-2 cell line and transfer RNA served as positive and negative controls respectively. Sizes of protected fragments were calculated relative to restriction fragments of known length (track M) and a standard DNA sequence ladder (indicated as TCGA).

Table 3. Association of INT2 *amplification with clinicopathological parameters*

	Not amplified	*INT2* amplified	Percentage amplified	*P*-value
Age				
≤ 50	44	8	15	
51–70	78	14	15	0.9
> 70	33	6	15	
Menstrual status				
Premenopausal	44	9	17	0.8
Postmenopausal	111	19	15	
Extent of disease				
Operable	119	23	16	
Locally advanced	29	3	9	0.7
Metastatic	7	2	22	
Nodal status (stages I/II)				
Node negative	39	8	17	1.0
Node positive	62	12	16	
Tumour size				
≤ 2 cm	26	1	4	
2.1–5.0 cm	94	20	18	0.2
> 5 cm	35	7	17	
Histology				
Ductal grades I/II	59	13	18	
Ductal grade III	54	9	14	0.7
Lobular	22	3	12	
Other	20	3	13	
ER				
< 20 fmol/mg	54	1	2	0.001
≥ 20 fmol/mg	91	27	23	
PR				
< 20 fmol/mg	74	9	11	0.07
≥ 20 fmol/mg	70	19	21	

constant in all the samples analysed, confirming that equivalent amounts of total cellular RNA were added to each assay. The relatively weak signal obtained with the *INT2* probe is indicative of the low level of expression of the gene, both in tumour 123 and Tera-2 cells. This leads us to conclude that, with rare exceptions, amplification of the *INT2* gene is not accompanied by detectable expression of the RNA. Similar conclusions apply to the *HST1* gene, although our analyses of this gene have been less extensive (data not shown).

INT2 *amplification and clinicopathological parameters*

Although these data imply that none of the 11q13 markers examined here can have a direct influence on tumour development, they in no way negate the potential importance of the amplification. While efforts to identify the key gene in the amplicon are in progress, it is pertinent to ask whether amplification of this region of the genome correlates with other clinicopathological features. Since *INT2* remains the most consistently amplified marker, we compared *INT2* amplification in chi-square tests with each of the parameters listed in Table 1. As shown in Table 3, a highly significant association was observed between positive ER status and amplification of *INT2* ($P = 0.001$). Thus, 27 out of 28 tumours (96%) in which the amplification was detected were scored as ER-positive (≥ 20 fmol/mg) compared to only 63% of non-amplified cases.

In contrast, *INT2* amplification did not appear to correlate significantly with age, tumour size, extent of disease, axillary node status, histological type, histological grade or PR status, although a weak association with positive PR status ($P = 0.07$) should not be discounted. Since the patients in this study presented within the last two or three years, it is too early to draw significant conclusions regarding prognosis, but this issue is being closely monitored.

DISCUSSION

The notion that *INT2* or *HST1* may play a significant role in the pathogenesis of breast cancer, as has been implied in some reports, is clearly attractive. The over-expression of growth factors (or their receptors) would be an obvious way for the aberrant growth of tumour cells to arise, and the implicit involvement of extracellular components, in either an autocrine or paracrine loop, would offer hope for therapeutic intervention.

However, accumulated data from several independent studies, including those presented here, argue against involvement of these growth factors. Although there are sporadic tumours in which the *INT2* gene is clearly expressed, and similarly for *HST1*, expression is rare compared to the frequency with which the relevant DNA is amplified.

In our own studies, sensitive RNase protection assays have detected *INT2* expression in only one of 28 tumours, including 12 in which the gene is amplified. An obvious caveat is that the technique measures the average signal throughout a tumour biopsy; if expression were restricted to a minority of cells in the tumour, perhaps those at a particular stage of differentiation, then the signal could be diluted out. Similarly, expression could be a transitory event early in tumour development and no longer evident at the time of clinical presentation. Indeed, *in situ* hybridization approaches, which can effectively probe expression in individual cells, have encouraged a more optimistic interpretation [34, 35]. However, although a higher proportion of the tumours are reputed to express *INT2* and/or *HST1*, there is still no convincing link between the amplification of *INT2* or *HST1* and increased transcription.

Perhaps the most conclusive evidence against the involvement of these genes is the existence of rare tumours in which the amplified unit of DNA does not encompass all the markers. For example, among the 183 tumours analysed in this study, there was a single case (tumour 123) in which *INT2* and *HST1* were amplified while *BCL1* was not. Other laboratories have reported similar findings, as well as reciprocal cases in which *BCL1* is amplified and *INT2* is not [19, 20]. The hypothesis is therefore that an as yet unidentified gene, tentatively assumed to lie between *BCL1* and *INT2*, must be the key component in the amplicon. There is also a tacit assumption that the same gene is likely to be important in a variety of other malignancies in which amplification of 11q13 has been observed, most notably oesophageal carcinomas [13], squamous cell carcinomas of the head and neck [15, 16], and primary hepatocellular carcinomas [18].

While the identification of this gene remains a high priority, the more immediately relevant clinical question is whether the amplification of the 11q13 region distinguishes a separate category of tumour. We previously reported the analysis of 106 breast tumours in which the amplification of *INT2* did not correlate significantly with any other clinicopathological parameter [17]. However, now that this study has been refined and expanded, there is an obvious and statistically significant preponderance of ER-positive tumours among those showing the amplification ($P = 0.001$). Similar findings have recently been reported by other groups [21] (and personal communications from A. Borg, University of Lund and E. Schuuring, Netherlands Cancer Institute). The majority of breast cancers are ER-positive, and, as a whole, such patients fare better than ER-negative cases. However, the group remains significantly heterogeneous as regards response to treatment and clinical outcome [36]. Subdividing these patients on the basis of 11q13 amplification may identify a class for whom the prognosis dictates a different therapeutic strategy. In this regard, the analysis might prove a useful complement to that of the *HER2/neu* gene, where amplification/over-expression is most prevalent among ER-negative tumours [21, 37–39]. *HER2* status was assessed in 128 out of the 183 tumours in the present study, and co-amplifications of *HER2* and *INT2* was noted in only three cases. It is therefore possible that, with rare exceptions, the two types of amplification may be indicative of separate categories of tumour. An obvious focus for future research will be to follow the progress of the current group of patients and to draw together the observations from other independent studies.

1. Bishop JM. The molecular genetics of cancer. *Science* 1987, **235**, 305–311.
2. Dickson C, Deed R, Dixon M, Peters G. The structure and function of the *int-2* oncogene. *Prog Growth Factor Res* 1989, **1**, 123–132.
3. Peters G, Dickson C. On the mechanism of carcinogenesis by mouse mammary tumor virus. In Medina D, Kidwell W, Heppner G, Anderson E, eds. *Cellular and Molecular Biology of Breast Cancer*. New York, Plenum Press, 1987, 307–319.
4. Peters G, Brookes S, Smith R, Placzek M, Dickson C. The mouse homolog of the *hst/k-FGF* gene is adjacent to *int-2* and is activated by proviral insertion in some virally induced mammary tumors. *Proc Natl Acad Sci USA* 1989, **86**, 5678–5682.
5. Delli Bovi P, Curatola AM, Kern FG, Greco A, Ittmann M, Basilico C. An oncogene isolated by transfection of Kaposi's sarcoma DNA encodes a growth factor that is a member of the FGF family. *Cell* 1987, **50**, 729–737.
6. Dickson C, Peters G. Potential oncogene product related to growth factors. *Nature* 1987, **326**, 833.
7. Yoshida T, Miyagawa K, Odagiri H *et al.* Genomic sequence of *hst*, a transforming gene encoding a protein homologous to fibroblast growth factors and the *int-2*-encoded protein. *Proc Natl Acad Sci USA* 1987, **84**, 7305–7309.
8. Adelaide J, Mattei M, Marics I *et al.* Chromosomal localization of the *hst* oncogene and its co-amplification with the *int.2* oncogene in a human melanoma. *Oncogene* 1988, **2**, 413–416.
9. Casey G, Smith R, McGillivray D, Peters G, Dickson C. Characterization and chromosome assignment of the human homolog of *int-2*, a potential proto-oncogene. *Mol Cell Biol* 1986, **6**, 502–510.
10. Nguyen C, Roux D, Mattei M *et al.* The FGF-related oncogenes *hst* and *int.2*, and the *bcl.1* locus are contained within one megabase in band q13 of chromosome 11, while the *fgf.5* oncogene maps to 4q21. *Oncogene* 1988, **3**, 703–708.
11. Yoshida MC, Wada M, Satoh H *et al.* Human *HSTI (HSTF1)* gene maps to chromosome band 11q13 and coamplifies with the *INT2* gene in human cancer. *Proc Natl Acad Sci USA* 1988, **85**, 4861–4864.
12. Lidereau R, Callahan R, Dickson C, Peters G, Escot C, Ali I. Amplification of the *int-2* gene in primary human breast tumors. *Oncogene Res* 1988, **2**, 285–291.
13. Tsuda T, Nakatani H, Matsumura T *et al.* Amplification of the *hst-1* gene in human esophageal carcinomas. *Jpn J Cancer Res (Gann)* 1988, **79**, 584–588.
14. Varley JM, Walker RA, Casey G, Brammar WJ. A common alteration to the *int-2* proto-oncogene in DNA from primary breast carcinomas. *Oncogene* 1988, **3**, 87–91.
15. Zhou DJ, Casey G, Cline MJ. Amplification of human *int-2* in breast cancers and squamous carcinomas. *Oncogene* 1988, **2**, 279–282.
16. Berenson JR, Yang J, Mickel R. Frequent amplification of the *bcl-1* locus in head and neck squamous cell carcinomas. *Oncogene* 1989, **4**, 1111–1116.
17. Fantl V, Brookes S, Smith R *et al.* Characterization of the proto-oncogene *int-2* and its potential for the diagnosis of human breast cancers. In Furth M, Greaves M, eds. *Cancer Cells*. New York, Cold Spring Harbor Press, 1989, Vol. 7, 283–287.
18. Hatada I, Tokino T, Ochita T, Matsubara K. Co-amplification of integrated hepatitis B virus DNA and transforming gene *hst-1* in a hepatocellular carcinoma. *Oncogene* 1988, **3**, 537–540.
19. Ali IU, Merlo G, Callahan R, Lidereau R. The amplification unit on chromosome 11q13 in aggressive primary human breast tumors entails the *bcl-1*, *int-2* and *hst* loci. *Oncogene* 1989, 34, 89–92.
20. Theillet C, Adnane J, Szepetowski P *et al.* *BCL-1* participates in the 11q13 amplification found in breast cancer. *Oncogene* 1990, **5**, 147–149.
21. Adnane J, Gaudray P, Simon M-P, Simony-Lafontaine J, Jeanteur P, Theillet C. Proto-oncogene amplification and human breast tumor phenotype. *Oncogene* 1989, **4**, 1389–1395.
22. Machotka SV, Garrett CT, Schwartz AM, Callahan R. Amplification of the proto-oncogenes *int-2*, c-*erb*B-2 and c-*myc* in human breast cancer. *Clin Chim Acta* 1989, **184**, 207–218.

23. Bloom HJG, Richardson WH. Histological grading and prognosis in breast cancer: a study of 1409 cases of which 359 have been followed for 15 years. *Br J Cancer* 1957, 5, 173–183.
24. Reed KC, Mann DA. Rapid transfer of DNA from agarose gels to nylon membranes. *Nucl Acids Res* 1985, **13**, 7207–7221.
25. Church GM, Gilbert W. Genomic sequencing. *Proc Natl Acad Sci USA* 1984, **81**, 1991–1995.
26. Brookes S, Smith R, Casey G, Dickson C, Peters G. Sequence organization of the human *int-2* gene and its expression in teratocarcinoma cells. *Oncogene* 1989, **4**, 429–436.
27. Sakamoto H, Mori M, Taira M *et al.* Transforming gene from human stomach cancers and a noncancerous portion of stomach mucosa. *Proc Natl Acad Sci USA* 1986, **83**, 3997–4001.
28. Tsujimoto Y, Jaffe E, Cossman J, Gorham J, Nowell PC, Croce CM. Clustering of breakpoints on chromosome 11 in human B-cell neoplasms with the t(11:14) chromosome translocation. *Nature* 1985, **315**, 340–343.
29. Krissansen GW, Owen MJ, Verbi W, Crumptom MJ. Primary structure of the T3-γ subunit of the T3/T cell antigen receptor complex deduced from cDNA sequences: evolution of the T3-γ and δ subunits. *EMBO J* 1986, **8**, 1799–1808.
30. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* 1979, **18**, 5294–5299.
31. Smith R, Peters G, Dickson C. Multiple RNAs expressed from the *int-2* gene in mouse embryonal carcinoma cell lines encode a protein with homology to fibroblast growth factors. *EMBO J* 1988, 7, 1013–1022.
32. Saito H, Koyama T, Georgopoulos K *et al.* Close linkage of the mouse and human CD3γ- and δ-chain genes suggests that their transcription is controlled by common regulatory elements. *Proc Natl Acad Sci USA* 1987, **84**, 9131–9134.
33. Williams BP, Shipley JM, Spurr NK, Smith DR, Hayman MJ, Goodfellow PN. A human sequence homologous to v-*sea* maps to chromosome 11, band q13. *Oncogene* 1988, **3**, 345–348.
34. Theillet C, Le Roy X, De Lapeyriere O *et al.* Amplification of FGF-related genes in human tumors: possible involvement of *HST* in breast carcinomas. *Oncogene* 1989, **4**, 915–922.
35. Liscia, DS, Merlo GR, Garrett C, French D, Mariani-Costantini R, Callahan R. Expression of *int-2* mRNA in human tumors amplified at the *int-2* locus. *Oncogene* 1989, **4**, 1219–1224.
36. McGuire W. Prognostic factors in primary breast cancer. *Cancer Surveys* 1986, 5, 527–536.
37. Berger MS, Locher GW, Saurer S *et al.* Correlation of c-*erb*B-2 gene amplification and protein expression in human breast carcinoma with nodal status and nuclear grading. *Cancer Res* 1988, **48**, 1238–1243.
38. Guerin M, Barrois M, Terrier M-J, Spielmann M, Riou G. Overexpression of either c-*myc* or c-*erb*B-2/*neu* proto-oncogenes in human breast carcinomas: correlation with poor prognosis. *Oncogene Res* 1988, **3**, 21–31.
39. Zeillinger R, Kury F, Czerwenska K *et al.* HER-2 amplification, steroid receptors and epidermal growth factor receptor in primary breast cancer. *Oncogene* 1989, **4**, 109–114.

Acknowledgements—We are grateful to A. Borg and E. Schuuring for communicating their results prior to publication and to R. Rubens and S. Gendler for comments on the manuscript. We also thank J. Burchell for the reduction mammoplasty RNA, C. Croce for the *BCL1* probe, M. Crumpton for *CD3*γ, S. Goodbourn for γ-actin, P. Little for *HST1*, M. Hayman for *SEA*, N. Spurr for *pMS51* and M. Waterfield for *HER2*.

Eur J Cancer, Vol. 26, No. 4, pp. 429–432, 1990.
Printed in Great Britain

0277–5379/90$3.00 + 0.00

Children Fathered by Men Treated with Chemotherapy for Testicular Cancer

Yvonne D. Senturia and Catherine S. Peckham

In a study designed to assess the potential teratogenic effect of paternal chemotherapy, information was obtained on 131 children fathered by 107 men treated for metastatic testicular cancer. Of this group, first born children fathered by 96 chemotherapy patients were compared with 96 children fathered by matched controls. There was no excess of malformations (relative risk 1.0, 95% confidence intervals 0.41 and 2.40).

In addition, the rates for specific malformations in the total cohort of 131 children were compared with the general population. There were no significant differences from national rates although the rate for congenital heart disease was higher than expected.

Eur J Cancer, Vol. 26, No. 4, pp. 429–432, 1990.

INTRODUCTION

TESTICULAR CANCER is the most common malignancy in men aged 20–34 in England and Wales [1]. Cure rates have improved dramatically over the past 15 years due to the introduction of effective chemotherapy (see [2] for review). The effect of treatment on fertility and the identification of any risk to children fathered after chemotherapy becomes of crucial importance in a predominantly young patient population. It is now clear that a substantial proportion of men treated with platinum containing chemotherapy for testicular cancer recover spermatogenesis [3–5] and a proportion subsequently father children [6]. Since chemotherapy usually has a profound suppressive effect on spermatogenesis frequently with a delay in recovery from 1–3 years, it has been suggested that damage to the germinal epithelium may result in viable but defective spermatozoa capable of fertilizing the ovum but producing abnormalities in the foetus. Indeed, in some cases pregnancies have been terminated on the grounds that foetal malformations might occur.

Correspondence to: Y.D. Senturia.
Y.D. Senturia is at the Children's Memorial Hospital, 2300 Children's Plaza, Chicago, IL 60614, U.S.A. and C.S. Peckham is at the Department of Paediatric Epidemiology, Institute of Child Health, 30 Guilford Street, London WC1 1EH, U.K.