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GROWTH FACTOR EXPRESSION IN BREAST TISSUE

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Summary—We have studied mRNA levels for a variety of growth factors in biopsy specimens from malignant, benign and normal breast tissue. We found TGFb mRNA in all breast cancers and neoplastic breast tissues but the level of the TGFb mRNA were found to be higher in breast cancers ($P = 0.01$). TGFa mRNA was detected in a similar proportion of cancers as in neoplastic breast tissues but the TGFa receptor EGFR mRNA was detected in only 55% of breast cancers but in all non-neoplastic breast tissue tested. The presence of EGFR mRNA was invertedly related to oestrogen receptor status and coexpression of TGFa and EGFR was observed in 28% of carcinomas, and significantly more commonly in ER negative tumours ($P = 0.01$). PDGF a and b chain transcripts coexisted in all normal and malignant breast tissue. Insulin-like growth factor II mRNA was present in all 15 samples of non-malignant breast tissue but in only 11 of 21 (52%) of carcinomas.

INTRODUCTION

Many peptide growth factors are known to be capable of stimulating the growth of breast cancer cells *in vitro*. They include Transforming Growth Factor alpha (TGFa, Salomon *et al.* [1]), Epidermal Growth Factor (EGF, Osborne *et al.* [2]) and IGF I and II (Salomon and Perroteau [3]). Platelet derived growth factor is also capable of stimulating the proliferation of breast cancer cells.

TGFb1 mRNA has been detected in many tissues and TGFb1 and b2 are known to inhibit the proliferation of breast cancer cells *in vitro* (Knabbe *et al.* [4]).

In view of these observations we have investigated the occurrence of growth factor transcripts in normal, benign and malignant breast tissue. Preliminary findings have already been published (Barrett-Lee *et al.* [5] and Travers *et al.* [8]).

MATERIALS AND METHODS

Patients and samples

Samples were collected at surgery and immediately frozen in liquid nitrogen for 6–72 months. In all 69 breast carcinomas, 20 samples of benign breast disease and six samples of normal reduction mammoplasty specimens were examined. Patient details are shown in Table 1. In all cases we confirmed histological diagnosis and of the breast carcinomas, 78% were infiltrating ductal carcinomas and 13% were lobular cancers. There were two mucinous and two medullary carcinomas, one tubular and one capillary cancer. We also recorded the amount of tumour stroma. Patients in this group were followed up and median duration of follow up at the time of our recent report (Barrett-Lee *et al.* [5]) was 42.5 months.

cDNA probes

We have described previously the origin of the cDNA probes used and the labelling procedure (Barrett-Lee *et al.* [5]). Concerning the analysis of RNA, total RNA was extracted from 0.5 to 1 g of frozen tissue as previously described and we obtained polyadenylated mRNA by passaging through oligo (dT) cellulose. We used Biodyne A membranes for dot blot analysis, using a BioDot apparatus as previously described (Barrett-Lee *et al.* [6]). For Northern analysis we used 2.5 μg of poly(A+) mRNA per sample and resolved this in a formaldehyde agarose gel and blotted onto Biodine A membrane. The filters were pre-hybridized in 50% deionized formamide 0.1% sodium dodecyl sulphate (SDS), 5 × Denhardt's solution and 5 mM EDTA, 0.75 M NaCl and 50 mM NaH_2PO_4 and denatured sonicated salmon sperm DNA. The filters were then hybridized overnight with the same conditions as for the pre-hybridization with the addition of the

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Table 1. Characteristics of patients studied

Characteristic		TGFa mRNA +ve	TGFa mRNA −ve	TGFb mRNA Low + medium	TGFb mRNA High
T-Stage	T1	9	4	5	9
	T2	10	20	14	15
	T3	4	8	3	8
	T4	2	2	3	1
	NK[a]	3	4	5	2
Nodal involvement	+ve	10	15	13	11
	−ve	14	14	8	21
	NK	4	9	9	3
ER status	+ve	18	27	20	25
	−ve	10	11	10	10
EGFR mRNA	+ve	18	17	12	20
	−ve	9	20	17	12
	NK	1	1	1	3
TGFb mRNA[b]	Low	1	3		
	Medium	11	15		
	High	16	20		
Total		28	38	30	35

[a]NK = not known.
[b]TGFb mRNA: Low = 0 or +, medium = ++ or +++, high = ++++ or +++++ on hybridization intensity scale.
This table demonstrates the characteristics of the patient's breast carcinomas and relates these to transforming growth factor mRNA content.
Levels of growth factor mRNA determined as in "Materials and Methods".

specific cDNA probe. After hybridization filters were washed and autoradiography was carried out using Hyperfilm MP with intensifying screens at −70°C for 4–14 days. Quantification of mRNA was carried out by comparison with serial dilutions of the appropriate plasmid.

RESULTS

TGFa

Twenty-eight out of 66 carcinomas (42%) contained detectable transcripts. Of these 18 (64%) were also ER positive. The level of TGFa mRNA was low and virtually confined to infiltrating ductal carcinomas. All eight lobular carcinomas were negative for TGFa expression. Twelve fibroadenomas, seven biopsies from mammary dysplasia, the six biopsies of histologically normal breast were studied. These contained significant levels of TGFa transcript in 4/12, 5/7 and 2/6 cases, respectively. Levels were similar to those found in carcinomas.

Ten unselected breast cancer mRNAs were subjected to Northern analysis. The eight positive for TGFa contained a 4.8 kb transcript but one cancer also contained a 2.2 kb species.

We carried out Southern analysis on 11 samples of carcinoma and normal breast but none of the cancers showed amplification but one case of mammary dysplasia showed a 50–100 fold amplification as did one sample of normal breast tissue.

We examined the relationship between the TGFa mRNA content and relapse free survival and overall survival. Details of the patients are shown in Table 1, but as shown in Table 2, there was no relationship to prognosis.

EGFR

Thirty-five of 64 breast cancer (55%) contained detectable transcripts for EGFR. Eighty-five percent were only of low intensity. Of the 35 EGFR positive carcinomas 51% were ER positive but 90% of EGFR negative carcinomas were ER positive ($P = 0.0001$). In contrast all normal and benign breast tissue contained EGFR message. Both EGFR and its ligand TGFa were expressed simultaneously in 18 out of 64 (28%) of carcinomas and eight of these

Table 2. Growth factor expression and relationship to prognosis in breast cancer

Growth factor/receptor		No.	Obs[a]	Survival Exp	Survival P-value	Relapse free survival Obs	Relapse free survival Exp	Relapse free survival P-value
TGFa mRNA	+ve	28	7	7.95	0.56	12	15.75	
	−ve	38	7	6.05		20	16.25	0.17
TGFb mRNA	Low + medium	30	8	7.98	0.99	19	14.73	0.11
	High	35	5	5.02		11	15.27	
EGFR mRNA	+ve	35	6	6.31	0.86	16	16.72	0.79
	−ve	29	8	7.69		15	14.28	

[a]Obs = observed number of events; exp = expected number of events. P-values determined from life tables using the logrank statistic.

were ER positive. Conversely 20 out of 22 carcinomas negative for both proteins were ER positive carcinomas ($P = 0.01$). Three carcinomas, one fibroadenoma and one carcinoma cell line were subjected to Northern analysis and in all cases three hybridizing bands were seen of 10, 6.4 and 4.8 kb. Details of follow up were available in these patients and, as shown in Table 2, as yet there is no relationship to relapse free survival.

TGFb

All 65 carcinomas and 20 non-malignant breast tissues contained TGFb mRNA. There was a clear difference in the level of TGFb transcripts between benign and malignant samples since 35 out of 65 (54%) of breast carcinomas had high levels compared to only 3 out of 20 (15%) benign samples ($P = 0.01$). No relationship was seen with the ER status but when we examined nodal status and TGFb expression a clear relationship was demonstrated with tumours from node positive patients having a significantly lower TGFb mRNA level compared with node negative patients ($P = 0.05$). Polyadenylated RNA from three carcinomas was analysed by Northern hybridization. In all cases a TGFb transcript of 2.5 kb was found. No other band was detected. We examined the influence of tumour TGFb mRNA levels on the survival of breast cancer patients. Although patients with higher levels of tumour TGFb mRNA had slightly longer relapse-free survival, this difference was not significant at the present time. More patients tumours will be measured for this transcript.

PDGF

Platelet-derived growth factor a and b chain transcripts were present in all normal and benign breast tissues. The amount varied somewhat in carcinomas but there did not seem to be any difference between carcinomas that were positive or negative for ER. PDGF b chain was undetectable in 4 of 34 samples (12%) of carcinoma.

Northern hybridization showed three bands had hybridized with the a chain probe at 2.9, 2.4 and 1.8 kb and a single band had hybridized with PDGF b chain probe at 4 kb.

Insulin-like growth factor II

Insulin-like growth factor II transcripts were found in all 15 normal and benign tissues but in only 11 of 21 carcinomas ($P < 0.005$). In non-malignant breast tissue the amount of IGF II mRNA was much higher than that of other growth factors. The amount of IGF II mRNA in carcinomas was generally low with a hybridization intensity of less than three.

DISCUSSION

Our results show some differences in expression of growth factor mRNA between breast cancers negative and positive for ER and between non-malignant breast tissues and breast carcinomas. The most interesting features to emerge from these studies are firstly that TGFb mRNA is increased in cancer compared to non-malignant tissue and secondly that EGFR and TGFa coexpression is predominantly in ER breast cancers. The existence of this autocrine loop implies that TGFa may be important in growth regulation in this subset of cancers.

Although we have failed to reveal any significant correlation with growth factor transcript level and prognosis, we are increasing the number of tumours analysed in an attempt to answer this question.

An interesting feature of our study has been to show that TGFa expression was generally low or absent in infiltrating lobular breast cancers. Most of these tumours were also EGFR negative. Although this may be explained by the fact that these tumours are mainly ER positive, it does imply that different forms of breast cancer may be regulated in different ways.

Further studies recently performed (Gomm *et al.* 1990) have demonstrated the localization of TGFb to the stroma in histological sections of normal and breast cancer tissue, and its localization may be important in determining the stromal–epithelial interactions in normal and malignant breast.

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