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Acidic and Basic Fibroblast Growth Factors in Human Breast Tissue

J. Smith, A. Yelland, R. Baillie and R.C. Coombes

Previously we have reported changes in fibroblast growth factors (FGF) in conditioned medium (CM) derived from rat mammary tumours undergoing remission. We have used a similar approach to assay for the presence of FGFs in human breast tissue and cell lines. The majority of cancer tissues (35/50), benign tissues (8/9) and all cancer adjacent normal tissues (20/20) released heat labile, NR6 transforming activity which coeluted from heparin with acidic FGF (aFGF) at 0.9–1.1 M NaCl and was neutralised by antibodies to aFGF. The conclusion that the majority of breast cancers contain active aFGF was supported by immunoblotting. The CM of a minority (15/50) of cancers and one benign tissue had highly transforming activity for NR6 cells, and was mitogenic for a breast cancer cell line, was heat labile, and strongly heparin binding, eluting at 1.5–2.0 M salt. It was not immunoreactive with antibodies to aFGF, basic FGF (bFGF) or Kaposi's FGF (kFGF) and its activity was reduced by the presence of aFGF, suggesting competition for the same receptor. Very little aFGF was observed in the CM of these tumours, and neither aFGF nor other FGF activity was detected in CM of breast cell lines.

Key words: breast cancer, fibroblast growth factors, breast cancer cell lines, acidic FGF, basic FGF
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INTRODUCTION

HUMAN BREAST carcinoma is the most common solid tumour of females in the western world. An effective treatment regime of oestrogen receptor (ER)-positive patients involves the use of endocrine therapy. Unfortunately, in the majority of patients, endocrine therapy ultimately becomes ineffective and the cancer regrows [1]. This process may be mediated by changes in growth factor secretion or by loss or gain of their receptors. The fibroblast growth factor (FGF) family of growth factors, of which to date there are seven members, are important in

developmental processes where they play a role in mesoderm induction. FGFs have a broad target specificity, but of particular importance to breast cancer, which is primarily of epithelial origin, are several FGFs which now have been shown to act on epithelial cells. These include basic fibroblast growth factor (bFGF) [2] and keratinocyte growth factor (KGF) [3]. Kaposi's FGF (kFGF) has been found only in tumorigenic or undifferentiated embryonic tissues [4], and is the protein product of the *hst* oncogene which, together with *int-2*, has been found coamplified in some breast tumours [5].

bFGF and acidic fibroblast growth factor (aFGF) are unusual in that they have no signal peptide sequence, leading to speculation about a new method of secretion of such factors or indeed whether they can be secreted at all. However, it is clear that both acidic and basic FGFs are found in conditioned medium (CM), in many different systems [6]. Sequestration from basement membrane or on cell death and rupture may also be important modes of release of FGFs, thus explaining the wound healing roles of both bFGF and aFGF. Both bFGF and aFGF are potent angiogenic factors, and may also be involved in basement membrane breakdown [7] and the stimulation of plasminogen activator, and thus could be important in invasion and metastasis [8].

Some FGFs act as transforming growth factors (TGF), and it may be the acquisition of a signal peptide sequence which enables these growth factors to escape the normal confines of the cell environment, and which facilitates their tumorigenic effects. TGFs (i.e. those growth factors able to induce anchorage-independent growth of certain cell lines) have long been implicated in tumour formation, and indeed, many were first identified from tumorigenic tissues [9–11]. However, their role and the mechanisms by which they exert their effect are not well understood. aFGF, bFGF and kFGF induce anchorage-independent growth of an epidermal growth factor (EGF) receptor-defective cell line (NR6) [12], when cultured in soft agar [4, 13, 14], whereas *int-2* (Dr C. Dickson, ICRF, London, U.K.), KGF (Dr J. Rubin, NIH, U.S.A.), TGFα, TGFβ and EGF do not [14].

We have used the transforming properties of the FGFs to screen breast cancer and non-cancer tissues for the presence of bioactive FGF-like peptide(s). We were able to subdivide these patients into groups according to the kind of FGF-like activity released by their tumours.

PATIENTS AND METHODS

Patients

Patients were those attending St George's Hospital breast clinic, and tissue was received from the pathology department within 30 min of surgical resection of their tumour. Tissue was then either placed directly into liquid nitrogen for the preparation of frozen sections or messenger RNA, or was placed into serum-free α-Eagles medium and removed directly for microdissection and preparation of CM, protein extracts for immunoblotting and paraffin sections. We have assayed CM and protein prepared from 50 breast cancer biopsies, the adjacent non-malignant tissues of 20 of these, and nine benign (fibroadenoma and reduction mammoplasty) tissues. Clinical details of the patients are shown in Table 1.

Preparation of CM

Tissue was collected into serum-free culture medium (α-Eagles) immediately after surgery and pathological assessment and was then removed directly for preparation of CM using a previously published method ([14] modified). Tissue was microdissected into cubes of approximately 400 μm/side, which were placed (two pieces per well) into the centre 60 wells of a 96-well tissue culture plate containing 100 μl/well serum-free α-modification of Eagles medium supplemented only with penicillin (100 U/ml), streptomycin (0.1 μg/ml) and glutamine (2 mM). Medium was collected after 24 h and then replaced for a further 72 h of incubation (37°C/5% CO_2). CM was then centrifuged (4000 rpm for 15 min at 4°C) and stored at −40°C. Serum-free controls were prepared following the same method and omitting tissue.

In order to ensure that activities measured were related to the source of tissue rather than the number of cells present or to variation in the preparation of CM, we monitored both cellularity of the source tissue and protein content after preparation of CM. Estimation of the amount of tissue used to prepare CM was achieved both by weighing and by assessing the protein content of the tissue remaining after collection of CM. Some variation in weight and protein was observed between tissue types and different batches of CM made from the same tissue type.

Table 1. Clinical details of patients with breast carcinoma and correlation with NR6-stimulatory activity

Clinical characteristics	All patients	Patients with aFGF-like CM activity only ('low activity')	Patients with BGF CM activity ('high activity')	
T stage				
T0–T2	21	11	10	$\chi^2=3.79$
T3–T4	13	9	4	$P=0.04$
Unknown	16	15	1	
Lymph node status				
Positive	13	7	6	$\chi^2=0.71$
Negative	12	7	5	$P=0.30$
Unknown	25	21	4	
Vascular invasion				
Positive	3	0	3	$\chi^2=7.74$
Negative	31	24	7	$^*P=0.0001$
Unknown	16	11	5	
Grade				
1	5	3	2	$\chi^2=5.62$
2	13	7	6	$P=0.06$
3	8	7	6	
Unknown	24	18	6	
ER status				
Positive	20	13	7	$\chi^2=7.15$
Negative	15	9	7	$^*P=0.005$
Unknown	15	13	1	
Histological type				
Infiltrating ductal	44			
Lobular	6			

Data was obtained from patients' notes by a clinician and correlations were determined by χ^2. * Indicates highly significant correlation. BGF, breast growth factor; aFGF, acidic fibroblast growth factor; CM, conditioned medium; ER, oestrogen receptor.

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However, these were not related to activity except at the very low extremes. Any tissue that fell outside 20% of either side of the mean was excluded. For estimation of cellularity and cell type, histological sections were made by standard haemotoxylin and eosin staining of 4 μm paraffin sections. A measure of cellularity was achieved by counting the cells in 10 randomly selected fields and analysing for intergroup variability using an unpaired *t*-test.

Detection of FGF bioactivity

Growth factor standards (bovine aFGF and bFGFs; British Bio-technology, U.K.) were diluted to stock solutions of 50 ng/ml and stored in aliquots at −40°C. CM (12 μl/well) or growth factor activities (1–5 ng/well) were assayed by their ability to induce anchorage-independent growth of NR6 cells as described previously [14]. Samples were placed in the centre 60 wells of a microwell plate (Sterilin) to which assay medium was then added containing 0.3% agar and α-Eagles medium supplemented with penicillin (100 U/ml), streptomycin (0.1 μg/ml), glutamine (2 mM), 10% fetal calf serum (FCS), 20 mM hepes buffer, $NaHCO_3$ and NR6 cells (2×10^3/ml). Cells were washed once in α-Eagles medium containing 5% FCS to remove trypsin before addition to the assay medium. All points were set up in fives with serum-free medium and FGF standard controls for each assay set. Heat treatment of CM was for 3 min at 100°C, and neutralising antibodies (British Biotechnology) were incubated for 1 h at room temperature with CM before assay. Controls and conditions have been described previously [14].

Immunoblotting and SDS–PAGE

Protein was extracted from frozen tissue samples of 38 human breast carcinomas, 21 cancer adjacent normal tissues, and from nine benign tumours and normal (reduction mammoplasty) breast tissue. Tissue was first homogenised and then centrifuged to remove fat and large particles, and samples stored in aliquots at −40°C. Protein concentration was determined using the method of Bradford [15], and samples (100 μg per track) were added to loading buffer containing β-mercaptoethanol and heated for 3 min at 100°C to complete reduction. SDS–PAGE was carried out as described by Laemmli [16] and blotted according to Khyse-Anderson [17]. Antibodies were used as follows: (a) mouse monoclonal anti-aFGF antibody (Upstate Biotechnology, U.S.A.) 4 μg/ml in 0.1% bovine serum albumin–phosphate buffered saline (BSA–PBS), (b) rabbit polyclonal anti-bFGF antibody 1 μg/ml as described previously by Gomm and colleagues [18], (c) rabbit polyclonal anti-mouse kFGF (a gift from Clive Dickson, ICRF, U.K.) at 8 μg/ml or, (d) rabbit polyclonal anti-mouse *int-2* (a gift from Clive Dickson, ICRF) at 8 μg/ml. All primary antibodies were incubated with blots overnight at 4°C, sealed in plastic bags. aFGF and bFGF antibody staining was visualised by the avidin/biotin-peroxidase method using an ABC vector kit (Vectastains, Vector Laboratories, Peterborough, U.K.) following the manufacturers instructions using 3,3′-diaminobenzidine (0.08%)/$NiCl_2$ (0.04%) as substrate. *Int-2* and kFGF antibody staining was also visualised by the more sensitive chemiluminescent detection method (Amersham, U.K.) following manufacturers instructions.

Heparin elution

One millilitre samples (CM, serum-free control, or aFGF and bFGF standards) were incubated with 50 μl sterile heparin-coated acrylic beads (Sigma, Poole, U.K.) for 1 h at room temperature (or 2 h at 4°C) on a rotating shaker. The absorbed beads were then removed by centrifugation and washed four times in PBS by centrifugation. The washed beads were then sequentially incubated (5 min) with increasing concentrations (0.3, 0.5, 0.9, 1.1, 1.5, 2 and 3 M) of tissue culture tested NaCl (Sigma), with PBS washes inbetween each incubation. Samples were then assayed on NR6 cells in soft agar as described above.

MCF7 cell proliferation assay

MCF7 cells were seeded into eight-well chamber slides (10^4 cells per well in a total volume of 400 μl per well) in phenol red-free Dulbecco Eagles' medium containing 10% serum-stripped FCS supplemented with insulin (10 μg/ml) and oestradiol (10^{-8} M) and allowed to attach for 5–6 h. Cultures were then refed with serum-free Dulbecco's medium (supplemented with insulin and oestradiol as before) and incubated (37°C/5% CO_2) until quiescent (20.5 h), when medium was replaced by either 2× CM (100 μl CM + 300 μl serum-free medium), 1 × CM (50 μl CM + 350 μl serum-free medium) or growth factors (1–10 ng/ml bFGF or aFGF; British Bio-technology) followed by a further 21 h of incubation. At this stage, [^{3}H]thymidine (Amersham) was added (1 μCi per well) for 7 h before processing for autoradiography as described previously [19].

RESULTS

Analysis of NR6 colony-stimulating activity in CM derived from human breast disease

CM was prepared from tumour biopsies of 50 breast cancer patients and from the adjacent normal tissue of 20 of these. CM was also made from nine benign biopsies (from fibroadenoma and reduction mammoplasty patients) and the adjacent normal tissue of three of the fibroadenomas.

CM from 35 of the 50 breast cancer biopsies (Figure 1a), all medium conditioned for 72 h (Figure 1b) and all of the adjacent normal, benign and adjacent benign tissue CM (Figure 1c), were found to stimulate low levels of NR6 cell colony formation in agar ('low activity CM'). Addition of aFGF increased colony formation up to but not above the plateau for aFGF (Figure 1 a–c), and heat treatment abolished nearly all of the NR6 stimulating activity (Figure 1).

The remaining 15(30%) breast cancer biopsies tested released a highly active and heat-labile FGF-like activity into CM after 24 h (Figure 1a). This activity, which we termed 'breast growth factor' (BGF), was characterised by its ability to stimulate NR6 cell colony formation well above the level of either bFGF or aFGF. Addition of aFGF reduced colony number (Figure 1a). We were, however, unable to reliably detect this activity in 72-h CM (Figure 1b). We found that this activity was highly labile (activity is lost within 4 weeks if CM is kept above −40°C). It was also very sensitive to freeze–thawing, and we found that one freeze–thaw cycle reduced the activity by approximately 50%.

Clinical correlations

Comparison of clinical data (Table 1) of patients with high and low NR6 cell colony-stimulating activity showed that there were no significant differences in the majority of clinical outcome indicators including clinical and pathological stage, histological type and lymph node status. We found significant differences in ER status ($P=0.005$), with an excess of ER-negative tumours in the 'high activity' group. However, the most striking correlation was that these patients were highly significantly more likely to have vascular invasion ($P=0.0001$). Vascular invasion was defined as the presence of cancerous cells in blood vessels in haematoxylin and eosin sections.

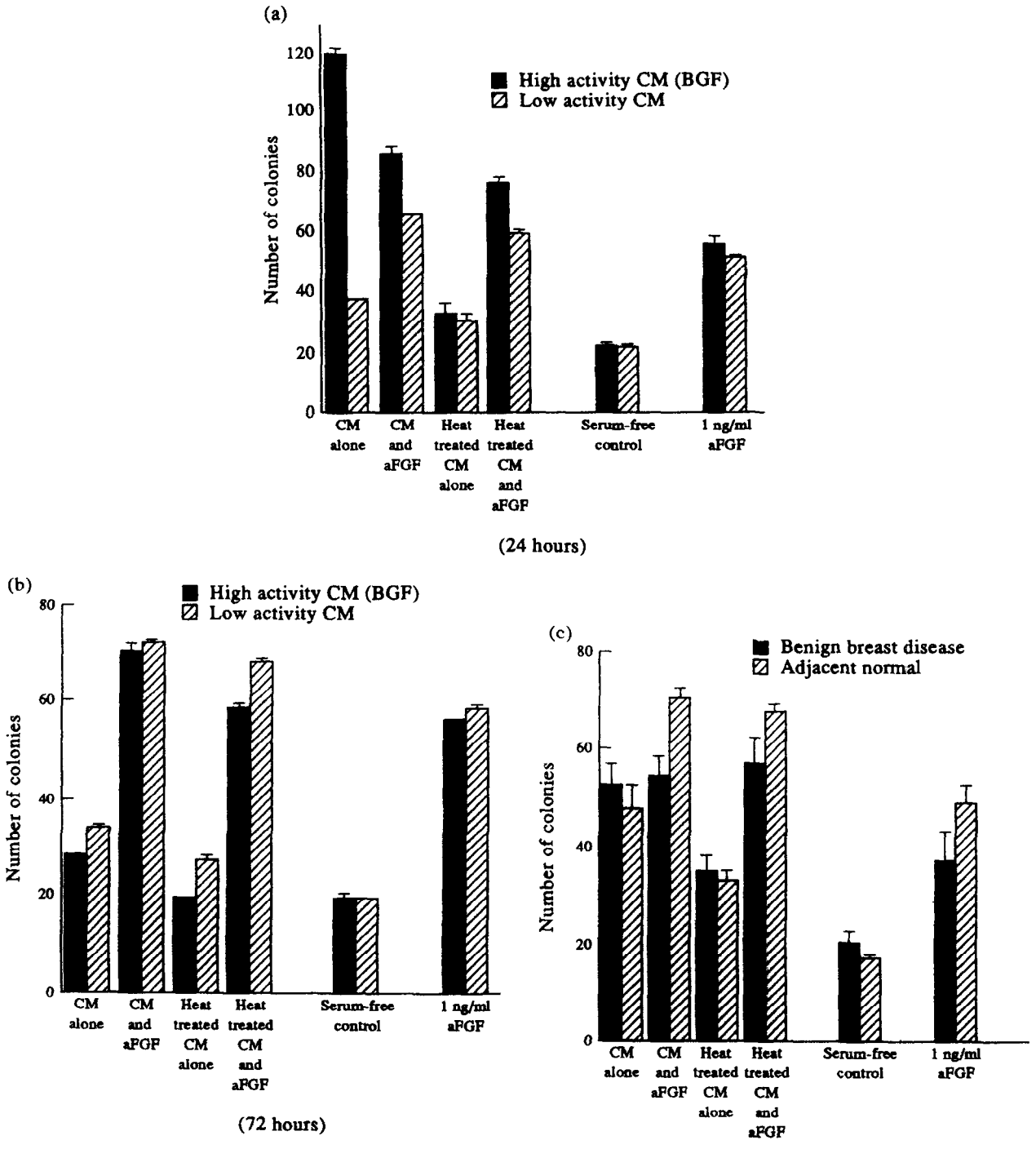

Figure 1. NR6-stimulating activity of (a) 24-h conditioned medium (CM) from 50 breast cancer patients, (b) 72-h CM of the same tissues (columns 1–4 as above), (c) 24- and 72-h CM of nine benign breast disease and 20 adjacent normal patients (columns 1–4 as above). Each assay was set up in groups of five and all patients CM was assayed in at least three separate experiments. BGF, breast growth factor; aFGF, acidic fibroblast growth factor.

The further division of 'high activity' patients into those with entirely heat labile and those with residual heat stable activity suggests that there may also be differences between the patients in these two subgroups (Table 2). The majority of patients in the high activity (stable) subclass were found to be both ER positive (six out of eight) and lymph node positive (four out of five known), both ER-negative patients had lobular disease. Of the high activity, heat labile group, 516 (known) were ER negative and none were lymph node positive.

The effect of breast cancer CM on MCF7 cell proliferation

Autoradiography of MCF7 cells incubated with tritiated thymidine and either aFGF or bFGF shows a dose-related increase in both cell number and labelling index, suggesting that both are mitogenic for this breast cancer cell line (Figure 2). Both aFGF and bFGF stimulate tritiated thymidine incorporation in quiescent MCF7 cells in a concentration range of 0–10 ng/ml, aFGF (36.6% at 10 ng/ml) and bFGF (32.9% at 10 ng/ml).

Breast cancer CM ('high-activity' group) was highly mitogenic

Table 2. Heat-stable activity in breast tissue conditioned medium (CM) assayed on NR6 cells in soft agar for anchorage-independent colony formation

Conditioned medium	Number of patients	CM alone	Heat-treated CM	Serum-free control
High activity (stable)				
(a)	8	110.1 (8.7)	45.6 (2.7)	21.1 (2.3)
(b)	8	28.4 (4.5)	18.7 (3.2)	18.8 (1.2)
High activity (labile)				
(a)	7	128.4 (8.5)	19.5 (5.9)	22.9 (3.3)
(b)	7	28.0 (1.4)	19.6 (0.5)	19.9 (0.8)
Low activity (stable)				
(a)	9	37.1 (1.2)	39.0 (2.1)	19.1 (2.0)
(b)	8	35.7 (2.3)	30.7 (3.7)	19.3 (0.8)
Low activity (labile)				
(a)	21	37.2 (2.4)	20.7 (1.4)	23.9 (2.0)
(b)	10	32.3 (1.8)	23.0 (1.9)	19.3 (1.1)

Each assay was set up in replicates of five and all patients CM was assayed in at least three separate experiments. (a) 24-h CM and (b) 72-h CM.

for MCF7 cells with a labelling index of 56.4% at a dilution of 1:4 (Figure 2). This compared with a labelling index of 29.8% for adjacent normal tissue CM at the equivalent concentration, which is consistent with the presence of small amounts of aFGF. CM derived from benign tissue was not found to be significantly stimulatory, 17.3% labelling index at the highest concentration, compared to a labelling index of quiescent cells in serum-free medium of 15.8%.

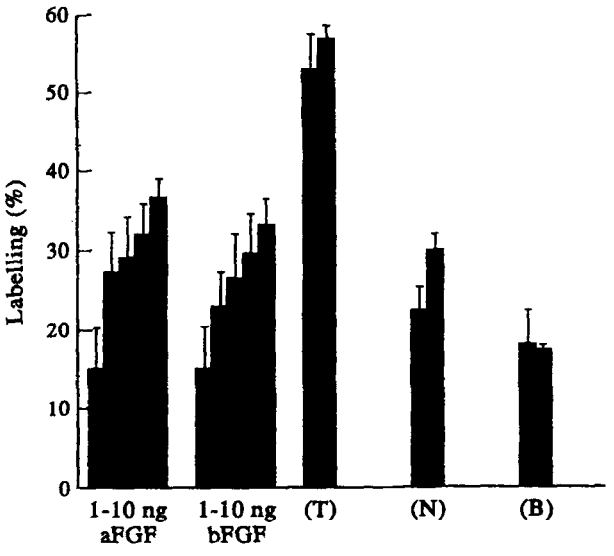

Figure 2. Autoradiography of quiescent MCF7 cells stimulated with medium conditioned with acidic or basic fibroblast growth factor (FGF); breast growth factor (BGF) tumour (T); adjacent normal tissue (N); or benign tissue (B) (fibroadenoma). First column, 1:8 (50 μl conditioned medium per 400μl well), second column 1:4 (100 μl conditioned medium per 400 μl well). Columns represent mean (±standard deviation) of tritiated thymidine-labelled nuclei calculated as a percentage of total cell count.

Heparin elution of breast tumour CM and salt elution of NR6 stimulatory activity: identification of aFGF

We salt-eluted the heparin binding activity of both low and high activity breast tumour CM, and aFGF and bFGF standards from heparin sepharose beads, and assayed the activity in each fraction on NR6 cells. We found aFGF eluted from heparin at 0.9–1.1 M NaCl while bFGF eluted at the higher concentration of 1.5 M NaCl (Figure 3a). Heparin elution of 'low activity' breast cancer CM (Figure 3b) primarily co-eluted with aFGF at 0.9–1.1 M NaCl, whereas NR6 stimulating activity from the 15 carcinomas with 'high activity' CM completely eluted in one peak at high concentrations (1.5–2 M NaCl), consistently higher than the elution of the bFGF peak at 1.5 M NaCl (Figure 3c). Seventy-two-hour CM activity also primarily co-eluted with the aFGF peak, although some activity was also observed in the 1.5–2 M fractions of some patient samples.

We were able to completely neutralise activity from the 0.9–1.1 M NaCl fraction of heparin-bound CM using neutralising antibodies to aFGF (Table 3). However, we found that we were unable to neutralise activity in the 1.5–2.0 M NaCl elution fraction with either bFGF or kFGF neutralising antibodies (Table 4), both of which are reported to elute from heparin at this salt concentration, nor with antibodies to aFGF. We were able to show that the peak eluted activity from bFGF and aFGF standards was neutralised by the appropriate neutralising antibody.

Immunoblotting of benign, normal and cancerous breast tissues for FGF-related proteins

We immunoblotted protein prepared from both normal and malignant breast tissues in an attempt to further characterise the activities that we had observed. We confirmed the presence of aFGF (Figure 4) in the majority (68%) of breast cancer samples (Table 5), and also detected aFGF in some adjacent normal tissues (57%). We did not detect aFGF in any of the benign or normal tissues assayed. Conversely, bFGF was present in all samples of normal and benign tissues that were examined, but not in any of the tumour samples assayed. kFGF was detected in only one tumour tissue and *int-2* was not found in any of the tissues assayed.

FGF activity in cell lines

We have assayed four breast cancer cell lines (two ER positive and two ER negative) and one immortalised normal breast line on NR6 cells but were unable to identify significant amounts of FGF-like activities in the CM of any of these cells (Table 6). As a control for the CM preparation technique we also tested CM from two non-breast cell lines in A204 and Nalm6. The rhabdosarcoma line A204, which expresses large amounts of FGF receptor mRNA, was found to release significant amounts of NR6 colony-forming activity likely to be one of the FGFs.

DISCUSSION

We have shown that bioactive FGFs can be found in human breast cancer tissues, and that they are mitogenic for at least one breast cancer cell line (MCF7). However, we have not detected FGF activities in significant amounts in the CM of any of the breast cell lines that we have assayed, suggesting that these cultured lines have lost some of the characteristics of primary breast cancers. Bioactive aFGF seems to be present in the majority of breast carcinoma tissues (70%) and in the adjacent normal tissue of these tumours, but was not found in any of the benign or reduction mammoplasty biopsies that we examined.

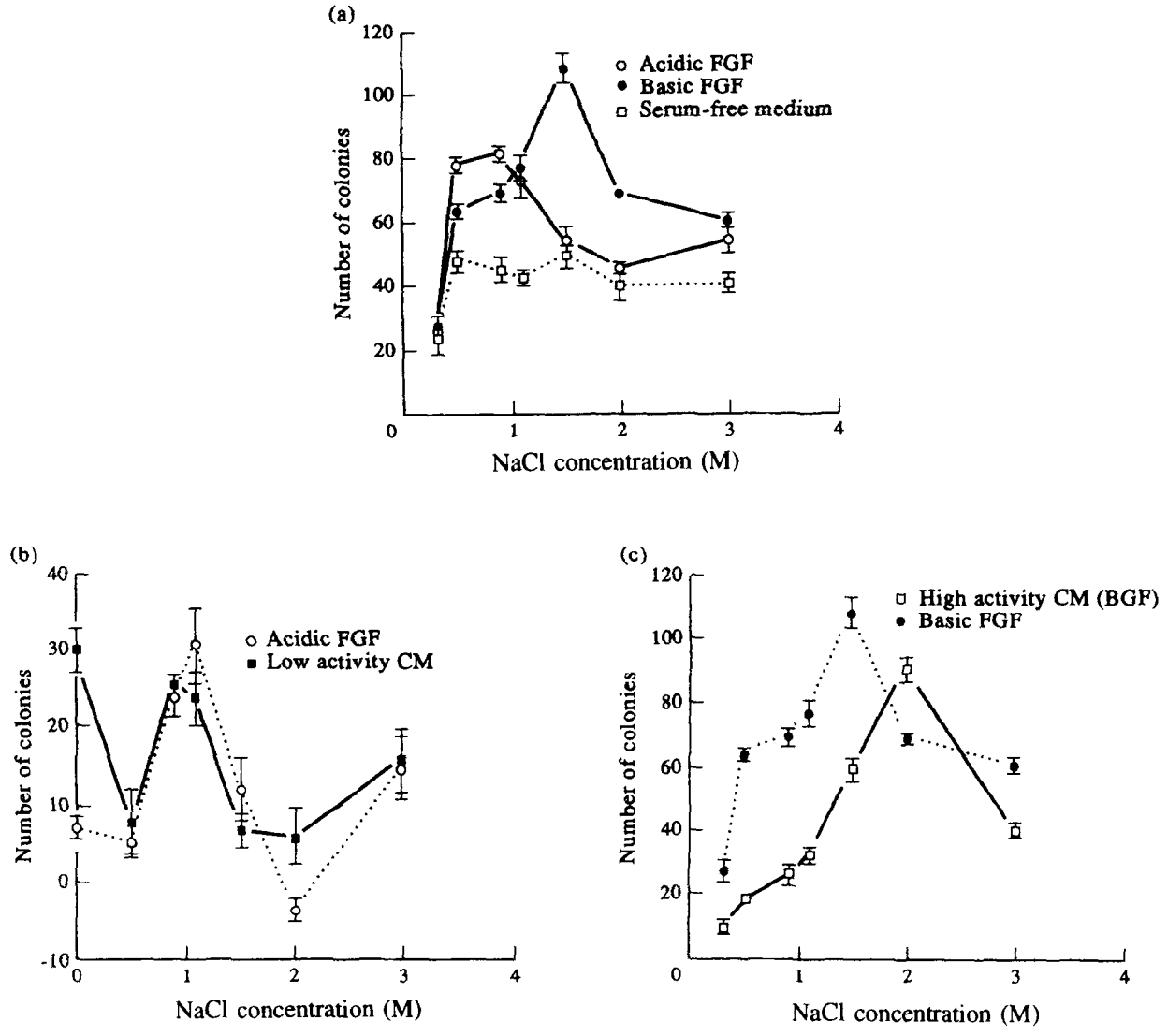

Figure 3. NR6-stimulating activity of salt-eluted fractions of heparin-bound conditioned medium (CM). (a) Acidic and basic fibroblast growth factor(FGF) standards compared with serum-free medium. Peak activities were obtained at 0.9–1.1 M NaCl and 1.5 M NaCl from acidic and basic FGF standards, respectively. (b) Heparin absorbed 'low activity' tumour CM compared with acidic FGF, demonstrating peak activity of both at 1.1 M NaCl. (c) Comparison of elution of basic FGF (1.5 M NaCl) with breast growth factor (BGF) obtained from heparin-bound 'high activity' CM, with peak activity at 2 M NaCl. Each point the mean (±standard error of the mean) of 10 data points.

Table 3. Neutralisation of tumour conditioned medium (CM) with fibroblast growth factor (FGF) antibodies. Heparin eluted fractions 0.9–1 M NaCl

	0 μg anti-acidic FGF antibody (control)	1 μg anti-acidic FGF antibody	10 μg anti-acidic FGF antibody	50 μg anti-acidic FGF antibody
Acidic FGF (0.9–1.1 fraction)	39.0 (0.6)	26.4 (2.5)	22.6 (2.1)	22.2 (2.0)
Breast carcinoma (tumour 0.9–1.1)	32.4 (2.4)	22.8 (2.1)	24.4 (1.6)	25.0 (1.9)
Breast carcinoma (adjacent normal 0.9–1.1)	35.8 (2.0)	27.8 (0.7)	25.4 (3.0)	25.2 (2.9)
Serum-free medium	22.8 (1.80)	23.4 (2.9)	23.4 (1.9)	22.0 (0.6)
Basic FGF (1.5–2.0 fraction)	49.4 (1.8)	52.2 (1.5)	49.4 (1.3)	51.0 (1.6)

aFGF antibody neutralised the NR6 colony formation activity of heparin eluted (0.9–1.1 M salt fraction) breast tumour and adjacent normal CM. No effect was observed on serum-free medium (negative control) or basic FGF (positive control).

Table 4. Neutralisation of tumour CM activity with FGF antibodies. Heparin eluted fractions 1.5–0 M NaCl

Tumour (1.5–2.0 fraction)	44.0 ± 4.4
Tumour (1.5–2.0) + 2 μl/well anti-kFGF	42.9 ± 3.9
Tumour (1.5–2.0) + 50 μg anti-bFGF	38.9 ± 3.5
Serum-free control	17.4 ± 2.1
Control + 2 μl/well anti-kFGF	17.8 ± 1.0
Control + 50 μg anti-bFGF	17.3 ± 1.8

Neither basic fibroblast growth factor (bFGF) nor Kaposi's FGF (kFGF) antibodies neutralise the activity eluting at 1.5–2.0 M salt.

Table 5. Immunoblotting of human breast tissue protein samples with fibroblast growth factor (FGF) antibodies, showing the percentage of the number of samples tested which were positive

Growth factor	Carcinoma	Adjacent normal	Benign disease
Acidic FGF	26/38 (68%)	12/21 (57%)	0/9 (0%)
Basic FGF	0/39 (0%)	20/20 (100%)	12/12 (100%)
Kaposi's FGF (hst)	1/31	0/11	0/3

Immunoblotting of breast cancer tissues showed a similar proportion of tumours (68%) with detectable aFGF protein. The slight reduction is probably due to the increased sensitivity of the bioassay, but the two findings are remarkably consistent. We have previously reported similar findings in regressing mammary tumours of nitrosomethylurea (NMU) treated rats [20].

Table 6. NR6-stimulating activity of conditioned medium derived from human breast and non-breast cell lines

Cell line	NR6 colony count (±S.E.)	Comment
Serum-free control	9.8 ± 3.5	Control
MCF7	11 ± 8.6	Breast cancer
ZR75	20.6 ± 8.5	Breast cancer
MDA	13 ± 5	Breast cancer
T47D	12.7 ± 2.4	Breast cancer
HBr	7.6 ± 8.4	Immortalised normal breast
A204	68.2 ± 6.2	Rhabdomyosarcoma
NALM6	12.2 ± 7.9	Leukaemia

We also found, in a third of patients, a highly active FGF which was found only in CM from malignant tissues, and was not found in adjacent normal or in benign tissue samples. This activity is heat labile and is more potent than either bFGF or aFGF in the stimulation of colony formation of NR6 cells in soft agar. This BGF activity is reduced in the presence of aFGF, suggesting that both factors compete for the same receptor. BGF is also highly mitogenic for MCF7 cells compared to both aFGF and bFGF, and appears to be associated with vascular invasion but not with any of the other clinical outcome factors analysed.

Our findings suggest that an increase in sequestration and possibly synthesis of aFGF may be an early feature of breast cancer development, and this is supported by the presence of aFGF in 'normal tissue' adjacent to tumour tissue but not in 'normal' reduction mammoplasty or benign tissues. Both our

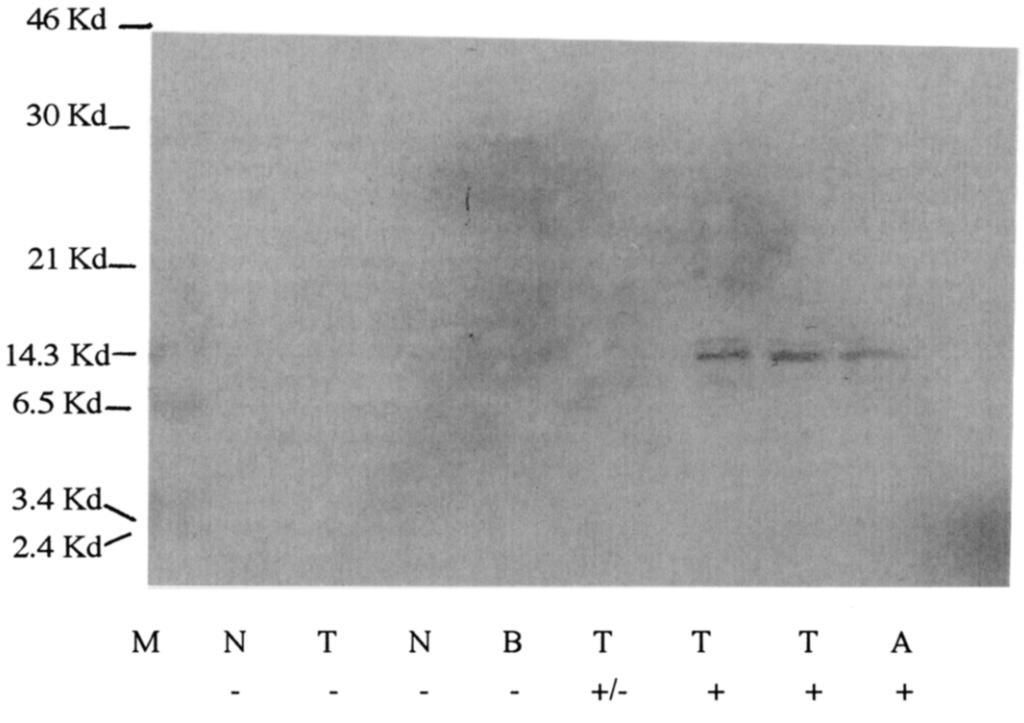

Figure 4. Immunoblotting of tumour and normal breast tissues for acidic fibroblast growth factor (aFGF) showing aFGF-positive (+) and aFGF-negative (−) cancers. N, 'normal' reduction mammoplasty tissue; T, cancer; B, benign fibroadenoma; A, aFGF; M, marker track.

laboratory [21] and others [22] have detected FGF receptors, FGFR-1 (FLG) and FGFR-2 (BEK), in human breast tissues, and Adnane and colleagues [22] reported amplification in some breast cancer tissues, suggesting an active role for the FGFs in breast cancer. A further two FGF receptors (FGFR-3 and FGFR-4) have also been described recently [23, 24], and have been shown to be expressed in some breast cancer cell lines including MCF7 [25], but have not yet been studied in breast tissues. Other receptors are also likely to be found. Previously, we reported bFGF as present in normal breast tissues associated with the myoepithelial cells of the basement membrane, and also in benign breast disease [18]. We also found that bFGF was not present in detectable amounts in any of the malignant tissues that we assayed. Valverius and colleagues [26] reported bFGF-like activity in the CM of normal mammary epithelial cells, further supporting the presence of this FGF in normal tissues.

One of the first morphological features of human breast cancer is the breakdown of the basement membrane and the loss of the myoepithelial cell component with which bFGF is associated. It may, therefore, be the case that the sequestration of other related growth factors, such as aFGF, or activation of the production of the signal peptide sequence coding embryonic FGFs, such as kFGF, are part of the survival strategy of such cancers.

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