Expression of basic fibroblast growth factor, FGFR1 and FGFR2 in normal and malignant human breast, and comparison with other normal tissues

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Summary The expression of basic fibroblast growth factor (bFGF) and two of its receptors, FGFR1 and FGFR2, was detected using the polymerase chain reaction, and quantified by comparison to the relative amount of product obtained following co-amplification of the ubiquitous glyceraldehyde phosphate dehydrogenase transcript. Varying levels were found in the vast majority of both cancer and non-malignant breast biopsies as well as in samples of several other normal human tissues. Significantly less bFGF was present in cancers (P<0.0001). Similarly, FGFR2 product was also much less in cancer tissues (P=0.0078), as was FGFR1 (P=0.002). FGFR1 levels in cancers tended to be higher in those which were oestrogen receptor positive (P<0.06). Amplification of different coding regions showed evidence of variant forms of FGFR1 RNA. Cancers appeared to have a significantly greater proportion of PCR product corresponding to the region between the third immunoglobulin like domain and the tyrosine kinase domain (P=0.046). Differential expression was observed in breast cell lines, with bFGF in the normal derived HBL100, HBR SV1.6.1 and 184A1 but little or none in ZR-75-1, MCF-7, T47D and MDA-MB-231. FGFR1 was present in most of these but FGFR2 was absent from T47D, MDA-MB-231 and HBL100. ZR-75-1 cells had a marked preponderance of FGFR1 variants lacking part of the coding sequence. Aberrant receptor processing may provide clues concerning the role of FGF's and their potential involvement in malignancy.

The fibroblast growth factors (FGF), acidic FGF, basic FGF, int-2, hst/k-FGF, FGF 5, FGF 6 and keratinocyte growth factor (Klagsburn et al., 1986; Gospodarowicz et al., 1986; Dickson & Peters, 1987; Tiara et al., 1987; Delli Bovi et al., 1987; Zhan et al., 1988; Rubin et al., 1989; Marics et al., 1989) are a group of structurally related heparin binding polypeptide mitogens of widespread tissue distribution that induce proliferation of most cultured cells derived from embryonic mesoderm and neuroectoderm, including endothelial cells. Sharing 33–55% amino acid sequence identity and similar genomic organisation, they are involved in differentiation, embryogenesis, angiogenesis, chemotaxis and wound healing (Baird et al., 1987; Burgess & Maciag, 1989; Rifkin & Moscatelli, 1989; Maxwell et al., 1991).

The int-2 and int-2 genes are co-amplified in a minority of human cancers, including breast (Thielt et al., 1989) but are poorly expressed. Acidic FGF expression is largely confined to neural tissues (Maxwell et al., 1991) whereas basic FGF is ubiquitously distributed in normal human tissues (Cordon-Cardo et al., 1990), and in neural and squamous carcinomas, melanomas, osteosarcomas and hemangiomas (Takahashi et al., 1990; Shulze-Osthoff et al., 1990). It is mitogenic for cultured mammary epithelial cells (Takahashi et al., 1989; Brozetto et al., 1991) and stimulates plasminogen activator (Lopez et al., 1986) which has been implicated in tumour invasion (Moscatelli & Rifkin, 1988). Basic FGF has been immunolocalised in myoepithelial cells in benign breast and around intraduct carcinomas but was not detected in cancer cells (Gomm et al., 1991).

The cellular response to these peptides is mediated through cell surface receptors composed of several extracellular immunoglobulin-like domains, a transmembrane region and an intracellular portion exhibiting intrinsic tyrosine kinase activity (Ruta et al., 1988; 1989; Lee et al., 1989; Safran et al., 1990). Several distinct human genes named fgr (FGFR1), bek (FGFR2), FGFR3 and K-sam (also FGFR2) bearing homology to the chick FGF receptor gene (Lee et al., 1989) have been described (Dionne et al., 1990; Keegan et al., 1991; Hattori et al., 1990). A fourth gene termed FGFR4 has also been reported (Partanen et al., 1991). For the FGFR1 (Fujita et al., 1991) and probably also the FGFR2 (Dionne et al., 1990), multiple forms of the receptor have been found comprising deletions within the extracellular domain, apparently generated by alternatively spliced mRNA's. Variant forms of the murine receptor appear to be developmentally regulated in neuroepithelium (Reid et al., 1990).

In view of the diversity of action of the FGF peptides and the apparent complexity in the processing of receptor isoforms, there is considerable potential for their involvement in malignant transformation, as well as in normal cellular interactions. In this report we describe the results of a study designed to assess the expression of bFGF and of the FGFR1 and FGFR2 receptors in human breast tissues. We have compared the levels of these mRNA's in normal/benign and cancerous biopsies with cultured cell lines and a variety of normal human tissues. Our findings indicate widespread expression of bFGF, FGFR1 and FGFR2, and suggest that many of these tissues contain variant FGFR1 receptor mRNA's in differing ratios.

Materials and methods

Chemicals

\[ ^{32} \text{P dCTP (3000 Ci mmol}^{-1}) \] was obtained from Amersham (UK), random hexamers, pdN, and dNTP's were from Pharmacia (Uppsala), MMLV reverse transcriptase was from Gibco BRL (Paisley, UK) and Taq polymerase was from Penninsula Laboratories (UK). Tissue culture media and foetal calf serum were from Gibco (Paisley, UK). All other reagents were obtained from Sigma (Dorset, UK) unless indicated and were of the highest available grade.

Tissue samples

Normal, benign and malignant breast biopsies were obtained from patients attending the Breast Clinics at St George's, the Royal Marsden and allied hospitals in London. No treatment had been given prior to surgery. Following histological con-
firmation of diagnosis, samples were dissected for material of interest and snap frozen and stored in liquid nitrogen. Tissues were examined from 66 primary carcinomas from patients aged between 29 and 79 years. Details of these patients are given in Table I. The non-malignant samples were composed of tissue removed from adjacent to cancer or from benign tumour. Other tissues used in this study were either surgical biopsy specimens or autopsy material.

Probes
The following cDNA inserts were used in this study: bFGF, 0.475 Kb excised from PBR322 (Abraham et al., 1986) FGFR1, 1.2 Kb excised from pGEM1 (Ruta et al., 1988) and FGFR2 excised from pGEM1 (Dionne et al., 1990). Hybridisations were initially performed using these clones, but after the identity of the PCR products had been independently verified (i.e. by restriction enzyme analysis), we used PCR products for labelling.

Cell culture
The following breast cell lines (Engel & Young, 1978) were maintained in continuous culture in Dulbecco’s minimal essential medium containing 10% primary carcinoma serum: MCF-7 (Michigan Cancer Foundation USA), T47D (Dr H. FreaKe), ZR-75-1 (Dr M. Lippman) MDA-MB-231 (Mason Research Institute, Rockville, MD, USA), HBR SV1.6.1 (Dr M. O’Hare), and HBL100. Cell pellets of 184A1 and 184B5 breast lines (Stampfer & Bartley, 1985) were provided by Dr M. Stampfer. The gastric line, KATO 111, was from Dr T. Motoyama and was maintained in RPMI-1640 medium containing 10% foetal calf serum. Cell pellets from squamous carcinoma lines, SMN, GEE and PAP were obtained from Dr B. Gusterson. The rhabdomyosarcoma cell line, A204, was from Dr C. Cooper.

RNA isolation
Total cellular RNA was extracted from frozen tissue using the guanidinium isothiocyanate procedure (Chirgwin et al., 1979), and a modified technique (Chomczynski & Saachi, 1987) utilising RNAZOL (Biogenesis, Bournemouth, UK) was used for cell lines. For Northern analysis, glyoxal-denatured RNA was electrophoresed on 1% agarose, trans- ferred to Hybond N membrane and hybridised with random primer labelled (Feinberg & Vogelstein, 1983) cDNA.

Reverse transcription
First strand cDNA was synthesised using MMLV reverse transcriptase. RNA (2 μg in 12 μl) was boiled, snap cooled and added to 1 μl of oligo (dT)20 units), 4 μl 5 × reaction buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, 15 mM MgCl2), 1 μl dNTP (20 mM each dATP, dCTP, dGTP and dTTP), 1 μl diethiothreitol and 1 μl random hexamers (250 ng). Following incubation at 37–42°C for 1 h, the mixture was heated at 95°C, snap cooled and stored at −20°C. With each experiment an additional tube which contained all the reagents except the enzyme was always included as a blank control.

Polymerase chain reaction amplification
Specific cDNA sequences were amplified (Saiki et al., 1988) in a reaction mix (100 μl) composed of 1–4 μl cDNA (equiva- lent to 100–400 ng RNA); 2 units Taq polymerase, 200 μM dNTPs, 200 ng each of the 5′ and 3′ sequence specific primers in various combinations and buffer containing (in final concentra- tions) 8 mM Tris-HCl pH 8.4, 40 mM KCl, 1.5 mM MgCl2 and 0.02% Tween, and overlayed with mineral oil. In the standard procedure, 18 cycles of amplification were performed with denaturation for 30 s at 94°C, annealing for 1 min at 45°C and extension at 72°C for 1 min with an extra 9 min extension for the last cycle. An aliquot (25 μl) was removed for measurement of GAP and the reaction continued for a further 12 or 22 cycles using the same parameters for the other gene products. All samples were analysed on at least two separate occasions to check for reproducibility. If there was more than 20% variation samples were re-done. In preliminary experiments to establish the conditions under which the levels in individual samples could be com- pared, the input cDNA was varied from 3–200 ng, and cycle number from 4–40.

Gel electrophoresis, blotting and hybridisation
Aliquots of chloroform extracted PCR products (10 μl) were electrophoresed in 1.5% agarose in Tris acetate EDTA buffer pH 8 containing ethidium bromide. Care was taken to ensure that exact amounts were loaded, this being essential for quantitive comparisons.

For blotting, gels were soaked briefly in 0.4 M NaOH, and DNA transferred onto Hybond N+ membrane in the same buffer. For hybridisation, filters were placed in roller bottles (Hybaid, UK) with a solution (60 μl cm−2) containing 50% (v/v) formamide, 0.1% SDS, 5 × Denhardt’s (0.1% each of polyvinylpyrrolidone, bovine serum albumin and ficoll), 5 mM EDTA, 75 mM NaCl and 250 μg ml−1 denatured sonicated salmon sperm DNA, and incubated at 42°C for 4–6 h. After this time, the relevant probe (either cDNA or PCR product) labelled with 32P-dCTP (to specific activities between 5 × 106 to 107 c.p.m. μg−1 DNA) using the random primer method (Feinberg & Vogelstein, 1983) was added and incubation continued for a further 16–20 h. Filters were subsequently washed in 2 × SSC, 0.1% SDS at 20°C for 25 min with five changes of buffer, and then at 60–65°C in 0.1 × SSC, 0.1% SDS for 1 h, with two changes of buffer, and exposed to Amersham hyperfilm at −70°C using intensifying screens for periods between 30 min to several days. Band intensities were quantified using a laser densitometer.

Calculation of results
To compare the levels of expression of the bFGF, FGFR1 and FGFR2 genes in different samples, the densitometric readings were expressed as a ratio of the signal obtained for

| Characteristic          | No. | %   |
|-------------------------|-----|-----|
| Total no.               | 66  |     |
| Age range               | 29–79 |    |
| Mean age                | 65  |     |
| Menopausal status       |     |     |
| pre                     | 15  | 31  |
| post                    | 33  | 69  |
| not known               | 18  |     |
| T stage                 |     |     |
| T<sub>0</sub>           | 2   | 4   |
| T<sub>1</sub>           | 15  | 33  |
| T<sub>2</sub>           | 20  | 43  |
| T<sub>3</sub>           | 6   | 13  |
| T<sub>4</sub>           | 3   | 7   |
| not known               | 20  |     |
| Histological node status|     |     |
| negative                | 30  | 68  |
| positive                | 14  | 32  |
| not known               | 22  |     |
| ER status               |     |     |
| positive                | 17  | 61  |
| negative                | 11  | 39  |
| not known               | 38  |     |
| Pathological size (mm)  |     |     |
| 10–20                   | 21  | 55  |
| >20                     | 17  | 45  |
| not known               | 28  |     |
| Histological type       |     |     |
| infiltrating ductal     | 36  | 90  |
| infiltrating lobular    | 4   | 10  |
| other                   | 4   |     |
| not known               | 22  |     |
GAP, a ubiquitously expressed gene encoding a common glycolytic enzyme. These values were normalised against the value obtained for A204 (for bFGF and FGFR1) or KATO 111 (for FGFR2) cells, a sample of which was included on every run and was present on each blot. The final value was calculated as below:

\[
\text{Signal for FGFR (sample)} = \frac{\text{signal for GAP (sample)}}{\text{signal for GAP (A204)}} \times \text{signal for FGFR (A204)}.
\]

* or KATO 111 as appropriate.

**Statistical tests***

In order to compare the two unequal groups of cancer and non-malignant tissues, both with skewed distributions, we used a non-parametric test, the Mann Whitney U test, which gave a two tailed probability. The data was also analysed by the Kruskal-Wallis one-way analysis of variance by ranks and gave the same probability values.

**Restriction enzyme analysis***

To verify identity of PCR products, a sample was ethanol precipitated following a phenol extraction, resuspended in water and digested for 3 h with the appropriate restriction enzyme. An aliquot was electrophoresed on 1% agarose and bands visualised with ethidium bromide.

**Oligonucleotide primers***

Oligonucleotides were synthesised on a Cyclone Plus DNA Synthesiser (Milligan Bioresearch, Massachusetts, USA) by phosphoramidite chemistry, deprotected by treatment with NH$_2$OH for 5–6 h at 55°C, vacuum dried, resuspended in water and used without further purification.

The primer pairs (Table II) were designed such that the intervening sequence was contained at least one intron. The functional domains of the FGFR’s encoding the regions amplified are shown in Figure 1.

**Results***

We were unable to detect the presence of mRNA for either FGFR1 or FGFR2 using conventional Northern blotting methods, with up to 20 µg of total RNA extracted from breast tissues, under conditions in which the appropriate bands could be seen with A204 or KATO 111 cells. Use of poly A* RNA could increase sensitivity, but tumour tissues generally yielded too little RNA for poly A* selection. We therefore used the PCR technique to perform this study.

**Optimisation of PCR conditions***

Figure 2 (inset) shows the accumulation of GAP PCR product with varying number of amplification cycles, using an input of 100 ng of RT product from the RNA of a tumour sample taken at random. Whilst ethidium bromide stained bands were visible after 20 cycles, product was detected after 12 cycles by hybridisation with 32P-labelled GAP cDNA, and increased linearly between 10–30 cycles. The mid-range, 18 cycles, was therefore selected for measuring GAP levels. Product accumulation was directly proportional to input template (3–200 ng RNA) after 18 cycles (Figure 2). The reproducibility of the method was assessed in several experiments in which the RT product from 2 µg RNA from 13

| Table II Oligonucleotide primers |
|----------------------------------|
| **RNA** | **Primer sequence** | **Product size (bp)** | **Reference** |
| bFGF | CTGATCAGCAGAAAAACGGG AAGGATATAGCCTTTCGCC | 349 | Abraham et al., 1986 |
| FGFR1 (A) | CTCCTCTGGGCTTGCT CCGGATACCTGTGGT | 433 | Dionne et al., 1991 |
| FGFR1 (B) | GCGGAGAAGTCGAGGTGTGCT GGTGAGAAGTTCCGACC | 801 | Dionne et al., 1991 |
| FGFR2 | GGTCTTATCAGCCTGCTG | 578 | Dionne et al., 1991 |
| GAP | TCCATCAACCACCCAGTTC | 379 | Arcari et al., 1984 |
samples was divided into three portions and GAP amplified (a), alone (b), in the presence of FGFR1 primers B or (c), in the presence of FGFR1 A and FGFR2 primers (Figure 4): the variation was generally no greater than 50%, and the yield of GAP product was not greatly affected by the presence of the other primers. The yield of product from the FGFR1 and FGFR2 primers was reduced when GAP primers were present; but independently of template. The bFGF primers would only work on their own. Thus the optimal primer combinations used were (a), GAP and FGFR1 B (b), GAP and FGFR1 A and FGFR2 and (c), bFGF alone. In the latter case it was necessary to amplify the GAP control separately, but to maximise uniformity, complete double volume PCR mixes containing cDNA were divided into two tubes containing the respective primers, and amplification performed in adjacent wells of the Thermocycler. Forty cycles of PCR were optimal for detection of FGFR1 and FGFR2 and 30 cycles were most suitable for bFGF (Figure 4), being within the linear stage of amplification. The slope of the linear part of the curve for each primer set was very similar, suggesting similar amplification efficiencies under the particular conditions we used. It should also be noted that this linear amplification was occurring at a time when GAP amplification had plateaued after 30 cycles, in the same reaction mixture. Thus it is valid to sample different PCR products at different cycle numbers.

For all the genes concerned we verified the identity of the product both by demonstrating hybridisation to the appropriate cDNA and by site specific cleavage with restriction enzymes (data not shown).

**Expression of bFGF in breast tissues**

All 66 biopsies taken from patients with primary breast cancer, and 28 samples of either histologically normal tissue taken from adjacent to cancer or from patients with benign disease produced the expected 349 bp bFGF PCR product but the relative amounts varied greatly within both groups (Figure 5). The mean ± s.e.m. for the cancers was 0.87 ± 0.12 as compared with 4.25 ± 1.03 for the non-malignant group; these being significantly different (P < 0.0001).

**Expression of FGFR2 in breast tissues**

As with bFGF the individual levels of FGFR2 PCR product varied considerably in both cancers and non-malignant tissues alike (Figure 5). Apart from the expected 578 bp fragment, hybridisation to two smaller bands was also observed; the origin of these is being investigated in the light of data which has appeared since this work was completed (Hou et al., 1991). Only in three samples of cancer (< 5%) was FGFR2 undetectable. Otherwise, there was a significantly greater level of amplified FGFR mRNA product in the normal tissues: means ± s.e.m. being 3.42 ± 0.46 and 9.92 ± 2.5 for cancers (n = 66) and non-malignant (n = 31) samples respectively (two-tailed probability = 0.0078).

**Expression of FGFR1 in breast tissues**

We used two sets of primers to amplify either the region encoding the outermost immunoglobulin-like domain, or that spanning the sequence from the third immunoglobulin-like domain to, and including, half of the tyrosine kinase domain. Again a wide range of values were recorded for both cancers and non-malignant biopsies, with both pairs of primers (Figure 5). With FGFR1 B primers the two groups had means ± s.e.m. respectively of 0.97 ± 0.11 (n = 66) and 1.78 ± 0.39 which were not significantly different. Product was obtained with all samples. With FGFR1 A primers the means ± s.e.m. were 3.96 ± 0.62 (n = 66) and 12.6 ± 2.6 (n = 31) for cancers and non-malignant groups respectively.
These two values were significantly different (two-tailed probability = 0.0002). In the cancers, two samples (<3%) had no detectable FGFR1 A product and three in the non-malignant group (10%). The relative amount of FGFR1 product varied considerably depending upon which region was being amplified. Thus, instead of the expected constant ratio for FGFR1 A: FGFR1 B product for all samples, we obtained widely different ratios. The mean values of the cancer and non-malignant groups were significantly different (means ± s.e.m. were 8.7 ± 1.5 (n = 63) and 46.9 ± 25 (n = 27) (two-tailed probability = 0.046).

**Relationship of bFGF, FGFR1 and FGFR2 expression to clinical parameters**

For those patients for whom clinical data was available, we analysed the results with respect to the major clinical features, T stage, nodal involvement and oestrogen receptor status. Table III shows the breakdown of this analysis, which was done by using a purely arbitrary cut off point to divide the tumour samples into two groups, with (comparatively) high and low levels of expression. The numbers are still too small to permit accurate correlate into clinical parameters, but oestrogen receptor positivity appeared to be associated with high expression of the FGFR2 mRNA. However, due to the small numbers, this relationship was only marginally significant (P<0.06, Fishers exact two-sided probability).

**Expression of bFGF, FGFR1 and FGFR2 in cell lines**

We examined RNA from four cell lines derived from breast cancer (ZR-75, T47D, MCF-7 and MDA-MB-231), four derived from normal breast cells (HBL100, HBRSV1.6.1, 184A1 and 184B5) and three from squamous carcinomas (GEE, PAP and SMN) (Figure 6). There was no bFGF product in three of the breast cancer lines, and only a small amount in ZR-75-1 cells. In contrast, all four of the ‘normal-derived’ breast lines had levels comparable with the low-range values found in cancer tissues. Of the three squamous lines two gave detectable bFGF product.

The FGFR2 product was present in MCF-7 and in ZR-75-1 cells but undetectable in T47D and HBL100 cells. The highest levels were seen in HBR SV1.6.1 and the 184B5 cells. Only PAP of the squamous lines produced FGFR2. Using FGFR1 A primers, highest levels were found in ZR-75 (in the same range as cancer tissues) with lower levels in HBL100, HBRSV1.6.1 and T47D. Product was barely detectable in MCF-7, and absent in 184B5 and 184A1. In the squamous lines expression was observed in GEE and SMN but not PAP cells. With the FGFR1 B primers, the pattern was similar except that there were very low amounts for ZR-75-1.

**Expression of bFGF, FGFR1 and FGFR2 in a panel of normal human tissues**

Basic FGF was present in heart, ileum, colon, kidney, stomach, adrenal gland, ovary, skin and thyroid with less than 5 fold differences between them, except for lung, which was much higher. The FGFR2 gene was also ubiquitously expressed, except in heart. The highest amount was present in the thyroid specimen.

All samples expressed FGFR1 with highest levels in skin ovary, and heart (Figure 7).

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**Table III** Relationship between bFGF, FGFR1 and FGFR2 PCR product levels and clinical status

| RNA | Relative level of PCR prod. | Total no. patients | Stage | Nodes | ER |
|-----|-----------------------------|--------------------|-------|-------|----|
|     |                             |                    | T3/T2 | T3/T4 |    |
| bFGF| ≤0.6                        | 36                 | 26    | 3     | 20 | 10 | 8 | 11 |
|     | >0.6                        | 29                 | 16    | 6     | 16 | 7  | 4 | 8  |
| FGFR1| ≤3                          | 39                 | 24    | 4     | 19 | 11 | 9 | 8  |
|     | >3                          | 27                 | 19    | 5     | 18 | 6  | 3 | 10 |
| (A) | ≤1                          | 39                 | 26    | 5     | 22 | 10 | 7 | 9  |
|     | >1                          | 27                 | 17    | 4     | 16 | 7  | 5 | 10 |
| FGFR1| ≤2                          | 38                 | 26    | 5     | 23 | 9  | 10 | 8  |
|     | >2                          | 28                 | 17    | 4     | 14 | 8  | 2 | 11 |

A and B refer to the primers used. Note that details not available for all patients.
Discussion

This study describes the expression of bFGF and two of the four recently identified FGF receptors, FGFR1 and FGFR2 in a series of human breast biopsies, cell lines and in a panel of normal tissues. Due to their low abundance, we devised a sensitive PCR based protocol which relies on co-amplification of an internal ubiquitously expressed sequence, and reference to values determined for an arbitrary standard sample. An advantage of using GAP, is that the level of this gene is likely to reflect the metabolic state of the cells and therefore its fluctuations would correct for differences due simply to differences in the proliferative activity, rather than specifically malignancy associated events. This procedure, similar to one described by Noonan et al. (1990) and unlike methods in which exogenously added cRNA is used (e.g. Wang et al. (1989)) overcomes the problems of variations in RNA quality and purity, sampling errors, and other differences in electrophoresis, gel blotting, hybridisation and autoradiographic exposure times. The main limitation is that our values are purely arbitrary and the actual levels of the three genes cannot be compared with each other. Although bFGF had to be amplified separately from GAP, we ensured that buffer conditions were identical and both tubes had exactly the same amount of cDNA. Duplicates done in this way showed no more than 20% variation.

In apparent contrast with earlier immunohistochemical observations (Gomm et al., 1991), we found that bFGF mRNA was present in breast cancer as well as benign tissue, though at significantly reduced levels. It is unclear whether this reflects the difference in sensitivity of the two methods, or differential translational control. All the breast cell lines derived from normal epithelia expressed the gene whilst only one of the four cancer derived lines did so and to a much lesser extent (more than 50 fold less). Valverius et al. (1990) reported the presence of bFGF mRNA in primary cultures of human mammary fibroblasts, but not in the transformed 184A1N4 epithelial cells. This discrepancy is probably due to the greater sensitivity of PCR over Northern blotting methods. We are currently doing cellular localisation using a combination of PCR with dissection of frozen tissue sections (Luqmani et al., 1992) to determine the precise cellular origin of the amplified RNA. The widespread presence of bFGF (Cordon-Cardo et al. 1990) suggests an important role in normal cellular function, but the lack of a signal peptide (Jaye et al., 1986), precluding secretion in the classically recognised manner, has posed problems regarding its potential as a locally produced para/autocrine factor. However its

![Figure 6](image1.png)  
Figure 6 Histograms showing the accumulation of PCR products from RT products of cell line RNA, for bFGF, FGFR1 (primers A), FGFR1 (primers B) and FGFR2 as indicated. Experimental details are described in Methods.

![Figure 7](image2.png)  
Figure 7 Histograms showing the accumulation of PCR products from RT products of RNA extracted from various normal human tissue samples, with bFGF, FGFR1 (primers A and B) and FGFR2 primers, as indicated. Experimental details as described in Methods.
appearance in conditioned media (Sato & Rifkin, 1988; Rifkin, 1991) and its association with the extracellular matrix (Saksela et al., 1988; Baird & Walicke, 1989) suggest that it may be externalised in conjunction with glycosaminoglycans which are also thought to modulate its biological activity (Ruoslahti & Yamaguchi, 1991; Yayon et al., 1991).

Both FGFR1 and FGFR2 were expressed in all tissues except heart (an observation also made by Kornbluth et al. (1991) and Reid et al. (1990)) and in the vast majority of the breast tissues. The wide range of values reflects the sensitivity of the PCR. In the light of growing evidence for the existence of truncated FGFR isoforms generated by alternative splicing, affecting all three structural domains, our results with the FGFR1 primers suggest that these commonly co-exist in the same tissue but in greatly varying proportions. Thus the ratio of PCR products obtained using the two FGF11 primer sets reflects a greater frequency of FGFR mRNA's with deletions in the outermost Ig like domains in the cancer tissues as compared with the non-malignant breast samples but that the truncated form is present in both groups. Fujita et al. (1991) observed this to be the major form in placenta and found that it still mediated biologic response to both acidic and bFGF, suggesting that this domain is not required for FGFR function, and play some other facultary role. Another placental cDNA encoding a soluble form of the placental receptor contains only the first two Ig like domains has been expressed in CHO cells and shown to undergo oligomerisation and retain binding activity (Duan et al., 1991). A truncated FGFR2 cDNA encoding only the signal peptide and the first Ig domain followed by a termination codon, and an FGFR1 clone with only the two outer Ig like regions have also been reported (Dionne et al., 1990). Spliced variants for the K-SAM (FGFR2) receptor have also been described in gastric carcinoma cells (Hattori et al., 1990). We found not only differential expression of FGFR2 and FGFR1 in the various cell lines studied, but also evidence of forms of the FGFR1 with deletions. ZR-75-1 cells appeared to have mRNA encoding only the external part. Hou et al. (1991) postulate the existence of up to 12 different variants of the FGFR1 receptor, which by extrapolation to the other three identified FGF receptors could extend this family to 48 isoforms. Johnson et al. (1991) have also recently described a number of different variants of FGFR1. The antisense primer of the FGFR1 A pair used in this study covers a region in which another variant involving a two amino acid deletion has been found: thus, lack of product with this primer may be due to such a variant in our samples, though it would have to be a predominant form. In the light of these recent findings, we are currently designing new primers to map these regions.

Other receptors also appear to display variant types. For example, developmentally regulated isoforms of the murine retinoic acid receptor beta generated by alternative splicing and differential promoter usage have also been described and may reflect functional specificities (Zelent et al., 1991).

The presence of receptors on several breast cell lines has been demonstrated by ligand binding studies (Peyrat et al., 1991; Biziozzo et al., 1991) and by Northern analysis, using 6 µg mRNA, in MCF7 cell (Lehtola et al., 1992). Mitogenic effects have been noted on both MCF-7 cells and on immortalised mammary epithelial cells derived from breast reduction mammoplasty (Valverius et al., 1990). Primary monolayer cultures of breast epithelial cells grown out of fragmented biopsy tissue were reported to show a modest response to bFGF (more pronounced in cancer than in non-malignant sample) which were unaffected by aFGF (Takahashi et al., 1989). As none of these studies were concerned with the identity of the receptor, it is not yet clear which of the sub-types are involved. Our results suggest that FGFR1 mediated events could be studied using T47D, MDA-MB-231 and HBL100 lines, all of which appear to be FGFR2 negative. The FGFR1 negative squamous line, PAP, could be used to study FGFR2 action. This of course does not take into account the presence of FGFR3 and FGFR4, the expression of which has yet to be determined.

A recent survey (Adnane et al., 1991) of 387 breast carcinomas showed amplification of both the FGFR1 and FGFR2 genes in about 12% of cases: FGFR1 amplification was correlated with nodal metastases and amplification of the HST, Int 2 and BCL 1 genes, and FGFR2 with c-myc.

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