Transforming growth factor β1 is implicated in the failure of tamoxifen therapy in human breast cancer

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Summary Transforming growth factor-β1 (TGF-β1) is inhibitory for breast epithelial cells in vitro and treatment of breast cancer cell lines with tamoxifen results in a rise in TGF-β1 mRNA expression with associated inhibition of cell growth. To study whether these findings apply in vivo we examined TGF-β1 mRNA expression in an oestrogen-dependent mouse xenograft system following systemic treatment of the mice with tamoxifen. In agreement with in vitro studies, TGF-β1 mRNA expression was sustained at high levels and associated with a reduction in tumour size. A subsequent study of breast tumour tissue from 56 patients demonstrated high levels of TGF-β1 mRNA in 45 of the tumours. High expression was found to correlate with premenopausal status, but not with tumour oestrogen receptor content or other parameters. In a subgroup of 11 patients who had received tamoxifen therapy for 3 to 6 months prior to surgery, unexpectedly high levels of TGF-β1 mRNA were demonstrated in tumours increasing in size and unresponsive to tamoxifen. Data from this study indicate that in patients with breast cancer, TGF-β1 in the tumour may not behave as in vitro and xenograft studies have suggested. We speculate that failure of tamoxifen therapy may be due to failure of the autocrine inhibitory functions of TGF-β1 either alone or in combination with paracrine stimulation of stromal cells or angiogenesis and localised immunosuppression. Further studies of active TGF-β1, TGF-β receptors and the interactions with other growth factors will be required to elucidate the precise role of TGF-β1 in human breast cancer and in the failure of tamoxifen therapy.

The transforming growth factor β (TGF-β) family are highly potent polypeptides first characterised from transformed fibroblasts (Derynick et al., 1985. At least three forms are now recognised, TGF-β1, TGF-β2 and TGF-β1.2 which result from various combinations of the 25 kd β1 and β2 subunits linked to form dimers by disulphide bonds (Chiefetz et al., 1987). With the three TGF-β cell surface receptors of 65 kd, 85 kd and 20 kd (Massague 1987), which bind the three forms of TGF-β differentially, a varied and complex series of distinct signals can be generated (Hsuan, 1989). This allows for different responses in different cell types and for flexibility of the regulation of tissue growth and differentiation by the TGF-β system (Chiefetz et al., 1987).

A role for the TGF-β family has been described not only in viral transformation of cells (Anzano et al., 1987) and carcinogenesis (Spor, et al., 1987) but also in embryonic development (Massague, 1987; Roberts et al., 1988). The wide range of possible biological activities for the TGF-β family is dependent on the cell type, the degree of cell differentiation and the other growth factors present (Roberts et al., 1985; Roberts et al., 1988). TGF-β can stimulate or inhibit cell proliferation depending on the experimental conditions or type of cells (Tucker et al., 1984; Roberts et al., 1985; Sporn, et al., 1987), with TGF-β1 and TGF-β2 strongly inhibitory on some epithelial derived cell lines (Tucker et al., 1984). For mammary epithelial cells in vitro, TGF-β1 is inhibitory for cell growth (Sporn et al., 1987; Knabbe et al., 1987; Lippman et al., 1987) with TGF-β1 ten times more active than TGF-β2 at inhibiting growth of the oestrogen dependent breast cancer cell line MCF-7 (Arrick et al., 1990).

TGF-β1 mRNA has been detected in a wide range of cell lines and tumour tissues (Derynick et al., 1985; Knabbe et al., 1987) with low or undetectable levels in normal tissue (Derynick et al., 1985) and higher levels in tumour when compared to adjacent normal tissues (Derynick et al., 1985; Coombes, 1989). In addition, the anti-oestrogen tamoxifen induces TGF-β mRNA production in vitro, but has no effect on the species of TGF-β mRNA expressed (Arrick et al., 1990) supporting the thesis that TGF-β1 stimulation is a mechanism of tamoxifen action against breast cancer cells (Knabbe et al., 1987). However, in apparent contradiction to this, MCF-7 cells transfected with the v-Ha-ras oncogene grew rapidly despite enhanced constitutive production of TGF-β1 (Dickson et al., 1987).

Given the interest which such in vitro studies have aroused and the possible involvement of TGF-β1 in the mechanism of action of tamoxifen, we sought to examine TGF-β1 expression in vivo, at the mRNA level, since detection of an mRNA implies dynamic production of the gene product. This paper presents studies of TGF-β1 mRNA expression in an oestrogen-dependent xenograft system, in human breast tumours and in breast tumours from patients who have received tamoxifen prior to surgery.

Materials and methods

Tamoxifen treatment of mouse xenograft tumours
An oestrogen-dependent xenograft system has been developed by injecting a single dose of 10⁵ viable MCF-7 cells into immune-compromised CBA mice (Thompson et al., 1990). The effect of tamoxifen treatment on tumour growth in this xenograft model was studied. A batch of ten mice each bearing on established single transplanted xenograft tumour of mean volume 250 mm³ received 50 μg 17β oestradiol benzoate in arachis oil injected subcutaneously into the groin as previously described (Thompson et al., 1990). This single injection is sufficient to sustain further xenograft growth for 3 weeks (Thompson et al., 1990). Tamoxifen (Nolvadex, ICI 46, 474) was dissolved in arachis oil and 1.25 mg injected daily, subcutaneously, into the hindquarter area, 2 cm from the xenograft. The dose of tamoxifen was calculated in milligrams of tamoxifen per kilogram body weight based on the human dose and tested for toxicity at 1.25 mg day⁻¹ on mice which had not received exogenous oestrogen. The series of tamoxifen injections commenced on the same day as the administration of the single dose oestrogen supplement. The tumours were measured daily using callipers, two mice killed and tumour material excised for study at 2, 4, 7, 14 and 21 days after commencing tamoxifen therapy. All tumours were snap frozen in liquid nitrogen for subsequent total RNA extraction after tissue had been removed for pathological examination and oestrogen receptor protein estimation.

Human tumour material
Fresh primary breast cancer tissue was obtained at operation from 56 patients with fully documented history, examination,
staging investigations and subsequent follow-up, who presented with operable breast cancer to the Department of Surgery Breast Unit at Longmore Hospital, Edinburgh. Of the 56 women (age range 34 to 84 years), 11 (all age over 70 years) required surgery due to failure of tamoxifen therapy (20 mg per day orally). These 11 tamoxifen-treated patients underwent mastectomy for tumour progression (increase in tumour size over a 3–6 month period; six patients) or lack of response to tamoxifen (tumour static in size over a 6 month period; five patients) assessed on clinical (caliper) and radiological grounds.

Tumour tissue from patients who underwent wedge biopsy, local excision or mastectomy as primary therapy for confirmed carcinoma of the breast, or following failure of tamoxifen therapy, was frozen in liquid nitrogen and stored at −70°C. Tissue immediately adjacent to that frozen was fixed for histopathology and a further piece of tumour submitted for oestrogen receptor assay. For comparison with constitutional DNA, 20 ml of venous blood was withdrawn for DNA extraction. Breast tissue from ten patients who underwent cosmetic reduction mammoplasty and who did not have a personal or family history of breast cancer was also obtained fresh and snap frozen.

The breast cancer cell lines MCF-7 (Soule et al., 1973), MDA-MB-231 (Cailleau et al., 1974) and T-47D (Keydar et al., 1979) were cultured and maintained under standard mycoplasma-free conditions (Barile, 1973). They were harvested in the logarithmic phase of growth and the total RNA was extracted for comparison with that from the tumours.

Ribonucleic acid extraction

From frozen tumour, total ribonucleic acid (RNA) was extracted using a modification of the method of Affurry and Rougeon (1980). Briefly, a known weight of frozen tumour or a known amount of cells washed in phosphate buffered saline was pulverised and then disrupted in 2 ml 3 M lithium chloride/6 M urea per 100 mg tissue and precipitated at 4°C overnight. The DNA was sheared using a Soniprep 150 ultrasonic disintegrator (MSE Scientific Instruments, Crawley, UK) with an ice jacket, the RNA was recovered by centrifugation at 12,000 r.p.m. and the pellet was taken up in 6 ml of 10 mM Tris buffer pH 7.0/0.1% sodium dodeyl sulphate (SDS). Three hundred micrograms of proteinase K was added and the sample was incubated at 37°C for 20 min. Protein was extracted using phenol equilibrated with tris (0.1 M, pH 7) and chloroformːisoamylalcohol (24ː1).

Following ethanol precipitation of the aqueous phase at −20°C, the RNA was recovered by centrifugation and dissolved in autoclaved distilled water treated with diethyl pyrocarbonate (DEPC, Sigma, USA) and stored in aliquots at −70°C. The quantity and purity of the RNA was assessed by spectrophotometry at 260 nm and 240 nm.

Throughout the RNA extraction procedures, sterile disposable plastic ware was used where possible; all solutions were made up with autoclaved, DEPC-treated water using baked glassware and gloves were worn to minimise exogenous ribonucleic contamination (Maniatis et al., 1982).

Electrophoresis and transfer of RNA

Twenty micrograms of total RNA was denatured with formamide and formaldehyde at 55°C for 20 min; 2 µl loading buffer (50% glycerol, 1 mM EDTA 0.4% bromophenol blue, 0.4% xylene cyanol) and 1 µl of 10 µg ml⁻¹ ethidium bromide were added to each sample. The denatured specimens were loaded onto a 1.1% agarose gel containing 0.66 M formaldehyde, submerged beneath MOPS buffer (morpholinopropanesulfonic acid 0.2 M, pH 7.0, 50 mM sodium acetate pH 7.0, 5 mM EDTA) and the RNA species were separated electrophoretically (method modified from Fourney et al., 1988).

The gel was washed in two changes of 10 X standard saline citrate (1 X SSC contains 150 mM sodium chloride, 15 mM sodium citrate, 1 mM EDTA, pH 7.4), photographed under a UV transilluminator and the RNA was transferred to a nylon filter (Hybond-N, Amersham, UK) by capillary action using 10 X SSC over 8 h (method modified from Southern, 1975). The filter was rinsed in 2 X SSC, air-dried and the RNA was covalently fixed to the membrane using a UV transilluminator. The filter and remaining gel were photographed to check for adequate transfer of the RNA.

Probe hybridisation

To detect the TGF-β1 mRNA, the 1.05 kb cDNA insert cut from plasmid sp65-C17N (Derynck et al., 1985) was used. Filters were prehybridised in 7% SDS, 0.5 M disodium hydrogen phosphate (pH 7.2) and 1 mMol EDTA pH 7.0 (method modified from Church & Gilbert, 1984) for 30 min at 65°C. To this was added 32P cytidine triphosphate (CTP) − labelled cDNA probe, with specific activity to 1 X 10⁷ c.p.m. ml⁻¹ achieved using a randomprime DNA-labeling system (Boehringer Mannheim, West Germany). 32P-CTP incorporated probe was separated from unincorporated radiolabel with a Sephadex column (Nick column, Pharmacia, UK) and denatured before addition to the hybridisation solution.

Following 24 h hybridisation, filters were washed to remove non-specifically attached probe in two changes of 0.1% SDS 10 mMol disodium hydrogen phosphate wash buffer at 65°C with agitation. The filters were blotted dry, wrapped in cling film and exposed to prefashed Kodak XAR film at −70°C overnight and then for up to 14 days.

The filter was washed clean of the TGF-β1 probe by incubation at 80°C in 0.1% SDS for 30 min and reprobed with a standard probe (the 1.4 kb PstI insert cDNA for actin mRNA; Minty et al., 1981), the expression of which is not affected by oestrogen (Saceda et al., 1988), to quantify the amount of intact mRNA present.

The extent of hybridisation of radiolabelled probe to the mRNA species was determined from laser densitometry and expressed with respect to hybridisation of the actin probe. The size of the TGF-β1 mRNA species was calculated from the position of ribosomal RNA markers.

Oestrogen receptors

The oestrogen receptor content was measured using the Enzyme Immunosorobent Assay (EIA; kit from Abbot Laboratories, North Chicago, Illinois; Hawkins et al., 1987) and expressed in fmol mg total protein⁻¹. Oestrogen receptor protein concentrations of 20 fmol mg protein⁻¹ or greater were considered to be clinically significant (Anderson et al., 1989).

Results

Tamoxifen treatment of MCF-7 xenograft tumours

Sequential xenograft tumour measurement demonstrated tumour regression over the 21 day period of tamoxifen treatment (Figure 1), rather than the expected increase in tumour size to approximately 800 mm³. Northern blot studies of TGF-β1 mRNA expression, as a percentage of actin control, demonstrated increased expression from very low levels at day 0 to a sustained, high level of expression from days 7 to 21 (Figure 1).

Human tissues

Intact mRNA for TGF-β1 of 2.5 kilobases (kb) in size was detected in all the tumour specimens and was also detected in normal breast tissues (Figure 2). High expression of TGF-β1 (>2 X that of normal breast tissue) was found in 45 of the 56 tumours (Table 1a).

Tumours from 18 of the 19 premenopausal women had
There was a significant correlation between high expression of TGF-β1 and premenopausal status (P = 0.05, Fisher’s exact test). There was no significant correlation between expression of mRNA for TGF-β1 and tumour size, tumour oestrogen receptor expression, or the presence of axillary nodal metastasis (Table 1a). Tumour tissue predominated over normal tissues in all the tumour specimens.

In the subgroup of 11 tamoxifen treated postmenopausal women (Table 1b), the six tumours increasing in size had high levels of TGF-β1 expression; 4 of the 5 static tumours had low levels of mRNA for TGF-β1.

In the six patients who exhibited breast tumours increasing in size despite tamoxifen therapy all the tumours expressed high levels of TGF-β1 mRNA in the tumour material and had oestrogen poor tumours (oestrogen receptor protein less than 20 fmol mg protein−1). From the five patients with tumours static in size following tamoxifen treatment, only one patient was oestrogen receptor poor and the same patient expressed high levels of TGF-β1 mRNA; the remaining four patients (who had oestrogen receptor moderate or rich tumours with greater than 20 fmol mg protein−1) had low levels of mRNA for TGF-β1.

### Table I TGF-β1 mRNA levels in breast cancer tissue in relation to clinical and pathological characteristics

| Characteristic                          | TGF-β1 Low | mRNA* | Probability (Fisher's Exact Test) |
|----------------------------------------|------------|-------|----------------------------------|
| (a) All patients (n = 56)              |            |       |                                   |
| Pre-menopausal (n = 19)                | 1          | 18    | 0.05                             |
| Pre-menopausal (n = 37)                | 10         | 27    |                                  |
| Tumour oestrogen receptor              |            |       |                                  |
| > 20 fmol mg protein−1 (n = 30)        | 7          | 23    |                                  |
| Tumour oestrogen receptor              |            |       |                                  |
| < 20 fmol mg protein−1 (n = 26)        | 4          | 22    |                                  |
| Tumour size > 5 cm mean diameter       |            |       |                                  |
| (n = 22)                               | 2          | 20    |                                  |
| Tumour size > 5 cm mean diameter       |            |       |                                  |
| (n = 34)                               | 9          | 25    |                                  |
| Histology involved nodes (n = 28)      | 7          | 21    |                                  |
| No node metastasis (n = 28)            | 4          | 24    |                                  |
| (b) Tamoxifen-pretreated patients (n = 11) |            |       |                                  |
| Disease static (n = 5)                 | 4          | 1     |                                  |
| Disease progressing (n = 6)            | 0          | 6     |                                  |

*Assessment based on intensity of TGF-β1 signal relative to that for actin. Specimens clearly fell into two groups, 50% of actin signal or less ('low') as in normal breast tissue and greater than 100% of actin signal ('high') on 24 h radiographs. BBased on men- strual history and, in perimenopausal women, on measurement of serum FSH and LH.

**Figure 1** Effect of tamoxifen treatment on MCF-7 xenograft tumour TGF-β1 mRNA. Tumour regression (solid line) following tamoxifen administration, associated with a sustained rise in TGF-β1 mRNA (hatched line). 17β oestradiol benzoate in arachis oil was administered at time = 0 (arrow); daily injection of 1.25 mg tamoxifen is indicated (T). TGF-β1 mRNA expression is expressed as a percentage of the actin mRNA signal from laser densitometry of the same Northern blot. Tumour volume was calculated from the equation: volume = πr/12 x mean diameter.

**Figure 2** TGF-β1 mRNA expression in human breast cancer. Autoradiographs of representative Northern blots from six human breast tumours. a, 2 breast tumours growing (i.e. uninhibited) on tamoxifen treated tumours and 4 breast cancer cell lines. b, MCF-7, c, T47-D, d, MDA-MB-231, left to right). The figure demonstrates the 2.5 kb TGF-β1 mRNA and the 1.8 kb actin signals for the same re-probed filter.

**Discussion**

We have examined TGF-β1 gene expression at the mRNA level in xenografts treated with tamoxifen and in 56 human breast tumours, 11 of which had been treated with tamoxifen prior to surgery. The MCF-7 oestrogen-dependent xenografts confirm the effect of tamoxifen on MCF-7 cells demonstrated in *vitro* (Knabbe et al., 1987). Tamoxifen treatment of the xenograft tumours was associated with a sustained rise in TGF-β1 mRNA to levels only briefly attained in the untreated tumours (Thompson et al., 1990) and a reduction in tumour volume.

We have confirmed that TGF-β1 mRNA can be isolated and quantified from human breast tissue and breast cancer cell lines and that the 2.5 kb TGF-β1 mRNA is the same size as that detected by other workers (Knabbe et al., 1987; Travers et al., 1988).

This study demonstrates that a high level of mRNA for TGF-β1 detected in breast cancer tissue correlates with premenopausal status but confirms that there is no associa-
tion with oestrogen receptor protein content (Travers et al., 1988). There is also an association between a high level of mRNA for TGF-β1 and progression of breast cancer despite tamoxifen treatment. On the basis of in vitro studies (Knabe et al., 1987) we had expected low levels of TGF-β1 gene transcription in tumours growing despite tamoxifen therapy, releasing the cells from proliferative constraints (Sporn & Roberts, 1985). However, high levels of TGF-β1 mRNA and clinically insignificant levels of oestrogen receptor protein (Anderson et al., 1989) were found in this group of human breast tumours. By contrast, patients in whom tamoxifen therapy had induced tumour stasis (but not regression) had low levels of TGF-β1 mRNA, but in most cases the tumour contained oestrogen receptor protein of greater than 20 fmol mg⁻¹ total protein. The association between high TGF-β1 mRNA expression and the failure of tamoxifen therapy may therefore be co-incidental given that these were oestrogen poor tumours. Against this proposition is the lack of correlation between TGF-β1 mRNA expression and oestrogen receptor protein, both in this work (Table 1a) and in one previous study (Travers et al., 1988), and the inhibitory effect of TGF-β1 on oestrogen receptor poor MDA MB 231 cells in vitro (Knabe et al., 1987). The data are also unlikely to be due to a direct effect of tamoxifen on TGF-β1 or due to TGF-β1 degradation (Knabe et al., 1987), although long term oestrogen deprivation of T-47D cells in vitro is associated with upregulation of TGF-β1 mRNA expression (King et al., 1989). Since tumour cells predominated over stromal cells and cells of haemopoietic origin, the difference between in vitro and human tissue studies are unlikely to be due to the presence of normal cells. However, in situ studies would demonstrate the distribution of the cells producing TGF-β1.

The unexpected finding of high TGF-β1 gene transcription in tamoxifen insensitive, growing breast cancers merits further consideration. We propose that two possible mechanisms may be involved: failure of the autocrine inhibitory feedback pathway on the cancer cells themselves and/or paracrine stimulation by the TGF-β1.

One mechanism for the escape from the autocrine inhibitory effects of TGF-β1 on the breast cancer cells (Figure 3) may be due to the failure to activate secreted TGF-β1 (Hsuan, 1989), over 98% of which is secreted in inactive from (Wakefield et al., 1987) an established control point for MCF-7 in vitro (Knabe et al., 1987).

Alternatively cells may fail to respond to the negative stimulus (Roberts et al., 1985) or respond weakly (Parkinson, 1985) due to reduction or loss of receptor function. The tumour cells may be resistant to the growth-inhibitory effects of TGF-β1 as has been demonstrated in vitro (Valverius et al., 1989) perhaps due to lack of functional TGF-β1 receptors as occurs in retinoblastoma (Kimchi et al., 1988).

There may be a mechanism similar to that observed following viral transformation of cells, where increased TGF-β1 secretion is associated with downregulation of the TGF-β1 receptors in the same cells (Anzano et al., 1985). However, transformation to full malignant potential and associated escape from TGF-β1 inhibition occurs without affecting TGF-β1 production or receptor characteristics (Valverius et al., 1989). Modulation of ligand-receptor binding may not be an important control mechanism in some systems (Wakefield et al., 1987), where modulation of TGF-β1 responsiveness may occur at the level of signal transduction (Valverius et al., 1989).

TGF-β1 may even directly stimulate the breast cancer cells, compatible with the in vitro observation that upregulation of TGF-β1 mRNA is accompanied by apparent stimulation (by the transcribed TGF-β1) of T-47D cells rendered steroid insensitive (King et al., 1989). It has also been proposed that tumour progression in one mouse mammary tumour model may, at least in part, be due to increased TGF-β1 expression (Cato et al., 1990). Thus one or more defects in the autocrine inhibitory TGF-β1 pathway may result in the failure of TGF-β1 inhibition on breast cancer cells in vivo.

A second, paracrine mechanism may also be involved (Figure 3). Stromal growth may be enhanced by increasing the levels of fibroblast mutagens such as interleukin 1 (Sporn et al., 1987). TGF-β species can greatly enhance accumulation of extracellular matrix (Massague, 1987), reduce proteolytic degradation by fibroblasts (Roberts et al., 1988) and increase the levels of mRNA for collagen and fibronectin (normal rat kidney cells, Sporn et al., 1987). TGF-β may act as chemotactic agents for macrophages (presumably stimulating them to secrete angiogenic peptides) and as potent stimulators of angiogenesis in vivo (Roberts et al., 1986; Sporn et al., 1987). In addition the local immunosuppressive effect of TGF-β1 (Kerhl et al., 1986; Sporn et al., 1987; Wann et al., 1987; Carey et al., 1990; Torre-Amoin, 1990) may also play a crucial role.

Given the mRNA and protein distribution of epithelial TGF-β1 (Akhurst et al., 1989), some combination of autocrine defect or paracrine effects on the supporting stroma may occur. The present study does not address the questions of TGF-β1 activation or receptor function which may be important in this as in other settings (Knabe et al., 1987; Hsuan, 1989). A further indication that the in vivo situation may be more complex than in vitro studies have suggested is the significant correlation between high TGF-β1 mRNA expression and premenopausal status identified in this study.

This may be explained by the local effects of TGF-β1, activity transcribed at a high rate in the breast tumour cells, dominating the effects of systemically circulating oestrogens which one might expect from in vitro studies to inhibit TGF-β1 transcription (Dickson et al., 1987).

There may also be different interactions between TGF-β1 and other growth factors (Roberts et al., 1986; Fernandez-pol et al., 1987) particularly epidermal growth factor and TGF-β1. Alpha also detected in breast tumour tissue (Travers et al., 1988). As additional considerations, TGF-β1 may be negatively regulated by binding to factors such as the proteoglycan decorin (Yamaguchi et al., 1990), itself induced by TGF-β1, or may act in a common growth inhibitory pathway with the retinoblastoma protein maintaining the protein in an underphosphorylated, growth-suppressive state (Laiho et al., 1990). Certainly, the effects of tamoxifen on breast cells ex vivo and in vitro are complex and other factors may well be involved (May & Westley, 1987; Johnson et al., 1989; Berry et al., 1989).

While the demonstrated inhibitory action of TGF-β1 on breast cancer cells and the effect of tamoxifen on TGF-β1 mRNA and cell growth in vitro are compatible with our findings in the oestrogen-dependent mouse xenograft system, the findings using human tissues merit further investigation. We propose that in tumours where tamoxifen fails to work in

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**Figure 3** A diagramatic representation of the autocrine and paracrine pathways for TGF-β1. The potential actions of TGF-β1 in breast cancer tissue: a, the autocrine inhibitory pathway whereby TGF-β1 produced by a breast cancer cell (as mRNA translated to protein precursor) is activated and acts via TGF-β1 receptors to inhibit the cell and, b, the paracrine stimulation of other cells and tissues or inhibition of immune response within the tumour.
vivo, there may not only be breakdown at one or more points of the TGF-β1 autocrine inhibitory loop, but additional paracrine effects mediated by TGF-β1, including stimulation of stromal growth and angiogenesis and escape from immunological surveillance. The net effect of tamoxifen in a subgroup of tumours may therefore be to stimulate tumour cell growth via the TGF-β1 system. With the recent demonstration that antiserum to TGF-β1 can suppress an experimentally induced disease state (Border et al., 1990), further analysis of TGF-β1 in breast cancer may identify potential therapeutic avenues. Certainly, the findings from this study suggest that the role of TGF-β1 in regulating the growth of human breast cancer, including tumour response to therapy, should be re-evaluated.

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