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Letters to the Editor

Changes in expression of transforming growth factor beta mRNA isoforms in patients undergoing tamoxifen therapy

Sir

The paper by MacCallum et al (1996) further investigates the role of transforming growth factor beta (TGF-β) isoforms in breast carcinogenesis and response to therapeutic intervention. Clinical material was restricted to patients over 70 years of age with oestrogen receptor (ER) positive tumours alledgedly at least 3 cm in maximum diameter. This may limit the value of the study and any conclusions drawn therefrom. Specific growth inhibitory effects of TGF-β are likely to be dependent upon stage of disease and may be operative only in early breast cancer with modest tumour load (Benson and Colletta, 1995). Presumably all these patients with relatively large primary tumours (? ≥4 cm) were free of overt metastases (Anderson et al, 1989). No comment is made on the accuracy of ultrasound alone in assessing tumour response and correlations between sonographic and pathological response criteria.

This paper nonetheless does rather tantalisingly allude to mechanisms for regulation of synthesis of individual TGF-β isoforms in response to anti-oestrogen therapy. Perry et al (1995) have recently cited data suggesting a mechanistic dichotomy, with dominance of post-transcriptional mechanisms at lower concentrations and transcriptional control at higher concentrations of tamoxifen. The present study provides further data in support of differential regulation. However, the authors appear diffident over whether they consider the observed variations in levels of TGF-β1 and TGF-β3 expression (which are statistically significant) to be a real effect or a consequence of a methodology that permits intratumoral assay variation of up to 100%. In the Discussion section, it is stated that there is no overall change in levels of TGF-β1 and TGF-β3 expression in response to tamoxifen. If there are in fact decreases in expression of β1 and β3 in some tumours but increases in others, it is inappropriate to draw any conclusions on mechanisms of control without some correlative data on corresponding changes in TGF-β protein. Immunohistochemical studies on this group of patients would have been useful; if levels of immunoreactive protein were to increase in tumours demonstrating either an increase or a decrease in TGF-β mRNA, then a complex interaction of both transcriptional and post-transcriptional mechanisms is implicit. If there are no overall changes in mRNA levels (with any apparent variations being attributable to experimental technique), then such results would be consistent with previous studies, and our own unpublished observations suggestive of post-transcriptional regulation of TGF-β. Arrik et al (1994) have reported the existence of two structurally distinct forms of TGF-β3 mRNA that display different rates of translational efficiency. Hence, alteration in the balance of these represents a further mechanism for controlling levels of protein synthesis independently of any overall quantitative changes in levels of transcript. Such issues introduce another dimension of complexity into the elucidation and understanding of the role of TGF-β in both neoplastic progression and mediation of the response to therapy.

Similarly, correlating changes in TGF-β2 mRNA expression with both immunohistochemical as well as clinical studies is desirable. As pointed out by the authors, previous work from our laboratory has demonstrated induction of the β1 isoform in response to primary tamoxifen therapy, but no significant enhancement of TGF-β2 was observed in either stromal or epithelial compartments irrespective of ER status (Butta et al, 1992). Interestingly, we have found significantly higher levels of TGF-β2 secretion in conditioned media of fibroblasts derived from benign rather than malignant breast tumours (Benson et al, 1996*a*). Malignant transformation may be associated with a selective reduction in β2 isoform expression which could be restored by pharmacological manipulation.

In situ hybridization studies have yielded mixed results; while some have shown epithelial cells to be the prime source of TGF-β (cited therein), others have indicated that TGF-β is located predominantly in the stromal compartment of primary breast tumours (Dalal et al, 1993; Kong et al, 1995). Failure to demonstrate increases in TGF-β expression in responsive tumours could be a consequence of exhaustion of stromal induction of TGF-β in these larger tumours with attenuation of negative paracrine effects. Larger more advanced tumours may exhibit epithelial expression of TGF-β, but this is more likely to be destined to promote stromal expansion rather than induce regression/apoptosis in these self-same cells (Benson et al, 1996*b*). Response of larger tumours to antioestrogens may principally involve classical ER-mediated effects. Functional redundancy amongst growth factors could undermine the influence of TGF-β as a negative growth modulator in this setting, with a poor correlation between clinical response and expression of TGF-β. Indeed, expression of TGF-β in epithelial cells could be suppressed as a secondary phenomenon to hinder stromal/angiogenic support and favour tumour regression.

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Changes in expression of transforming growth factor beta mRNA isoforms in patients undergoing tamoxifen therapy

Reply to the letter from Benson and Colletta

Sir

The controversy surrounding the complex role of transforming growth factor (TGF-β) in breast cancer is once again highlighted in the letter of Mr JR Benson and Dr AA Colletta. The comments made are informative and, while we would concur with many of them, we feel that some additional response is appropriate to clarify some of the issues raised.

First of all, it is important to emphasize that the aim of our study was to determine the effect of tamoxifen on TGF-β expression in breast cancers from patients whose tumours could be accurately assessed for response to therapy. It was not a study to elucidate the role of TGF-β in carcinogenesis or to assess expression in different stages of disease, although we have already published data on the latter (MacCallum et al, 1994). Although the study is limited to a defined group of patients, this is an important cohort, and one in which we could obtain sequential samples of tumour and accurately assess response of these same lesions to tamoxifen treatment (Incidentally, the rationale for using ultrasound as an accurate assessment of tumour size has already been published by Forouhi et al, 1994).

Secondly, we believe we have correctly shown conservatism in terms of attributing differences between sequential samples of the same tumour to the effects of tamoxifen. It is essential that inherent variations of methodology and tumour heterogeneity are assessed and realistically taken into account. Having done this in the present study, it was reassuring that, with regard to TGF-β2 not only was it more likely for expression to be higher in tamoxifen-treated biopsies but also that this pattern was exclusive to responding tumours. We have therefore been satisfied that these effects were mediated by tamoxifen. However, the direction of effects of treatment on TGF-β1 were almost equally increases or decreases, and there was no statistical difference in patterns between responders and non-responders. We have therefore been reluctant to claim dogmatically that these influences are caused by tamoxifen, despite a degree of change exceeding that of our controls.

Nevertheless, we have discussed the possibility that tamoxifen might more commonly induce the expression of TGF-β1 in breast cancers, an effect which may not have been apparent in our study for a variety of reasons (MacCallum et al, 1996). The exhaustion of stromal induction of TGF-β, as suggested by JR Benson and AA Colletta, is also possible. However, that the stroma is the primary source of TGF-β is controversial; both we and others have shown that TGF-β appears to be synthesized predominantly within epithelial cells of breast cancers (Auvinen et al, 1995; MacCallum et al, 1995; Walker and Gallagher, 1995). This is not necessarily at odds with the apparent increased staining of TGF-β1 in stroma following primary tamoxifen therapy, as reported by Butta et al (1992), if the growth factor was synthesized and secreted by epithelial cells, but sequestered by the stromal compartment. Indeed, if tamoxifen causes the death of epithelial cells, there might be an impression of upregulation in residual stroma. We would agree however, that simultaneous measurements of TGF-β protein and mRNA would give an additional dimension to these studies. Our immunohistochemical investigations are currently under way, and preliminary data suggest that both TGF-β1 and TGF-β2 predominantly localize to the epithelium.

In vitro studies using cell lines and animal models have yielded important understanding of the role of TGF-β in breast cancer, but ultimately it is necessary to look at appropriate clinical material. Such translational studies can be difficult to perform and may produce results that are subject to variable interpretation depending on perspective. However, in carrying out the reported study, we believe that we have not only generated meaningful results, but have been objective in deriving our conclusions.

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