

Epidermal growth factor and transforming growth factor α concentrations in BPH and cancer of the prostate: their relationships with tissue androgen levels

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Summary We measured immunoreactive EGF and TGF α in prostate tissue extracts obtained from 19 patients with benign prostatic hyperplasia (BPH) and 19 with cancer of the prostate (CaP). Whilst both BPH and CaP expressed EGF (BPH = 195.61 ± 19.94 ng g $^{-1}$ protein; CaP = 235.60 ± 24.45 ng g $^{-1}$ protein) and TGF α (BPH = 92.57 ± 7.60 ng g $^{-1}$ protein; CaP = 100.73 ± 15.47 ng g $^{-1}$ protein) in equal concentrations, the levels of EGF in any tissue extract were on average twice those of TGF α . Furthermore analysis of the individual growth factor data revealed a direct correlation between EGF and TGF α in both BPH ($r = 0.72$, $P < 0.001$) and CaP ($r = 0.69$, $P < 0.001$). When the tumours were classified according to their Gleason score, a slight but significant increase in growth factor concentrations was noted as the tumour became less differentiated. We also measured the concentrations of testosterone and dihydrotestosterone (DHT) in prostate extracts with a view of elucidating the relationship between androgen and growth factors in this gland. There was a small positive correlation only between testosterone and EGF ($r = 0.62$, $P < 0.05$) and testosterone and TGF α ($r = 0.61$, $P < 0.05$) in CaP. The absence of any similar correlation in BPH where DHT becomes the predominant hormone may suggest an indirect role for testosterone in the regulation of growth factor production.

Since the realisation that androgens are not alone in influencing the growth and regulation of the prostate, several investigations into the role of growth factors and their relationship with androgens have been undertaken. Initially, studies on the normal rat prostate demonstrated that the receptors for epidermal growth factor (EGF) were down regulated by androgens (Traish & Wotiz, 1987; St. Arnaud *et al.*, 1988). Subsequently experiments on androgen responsive prostate cancer cell lines revealed relatively low numbers of EGF receptors whilst androgen unresponsive cells expressed higher EGF receptor numbers (MacDonald & Habib, 1992). The correlation between EGF receptor expression and endocrine status suggest that steroid hormones might influence the response to growth factors, by altering growth factor receptor expression, but this response may also reflect the species from which the prostate cells were derived. Receptors for EGF have also been identified in human benign hyperplastic prostate (BPH) but their concentrations were markedly depleted in the poorly differentiated prostate cancer (Maddy *et al.*, 1989). Significantly, this loss in EGF-receptor expression is manifested at a time when the prostate tumour exhibits a reduced sensitivity to steroid hormones. It is not clear whether these two events are interrelated but the progression to an androgen independent state may, in part, be due to a reduced need for exogenous mitogens because of the the autologous production of growth factors (Sporn & Roberts, 1985). Transforming growth factor alpha (TGF α) is a polypeptide growth factor which has been reported to modulate autocrine growth in several tumour systems (Bates *et al.*, 1986; Hofer *et al.*, 1991; Lloyd *et al.*, 1992; MacDonald *et al.*, 1990) but no one has so far related the presence of this growth factor in BPH and cancer of the prostate to the tissular concentrations of EGF and prostate androgens. The purpose of this study is to investigate some of these relationships.

Materials and methods

Prostate tissue

Prostate tissue was obtained at the time of transurethral resection from 19 patients with BPH and 19 patients with prostatic cancer. The tissue was transported immediately to the laboratory chopped into 1–3 g pieces washed in 2×150 mm sodium chloride solution and dry blotted. Several sections from each specimen were sent for histological examination and grading of the cancerous tissue by the Gleason system (Gleason, 1977). The remainder of the tissue was snap frozen in liquid nitrogen and finally pulverised in a micro-dismembrator (Braun AG, Melsungen, Germany). The powdered tissue was then lyophilised to dryness and stored at -70°C until needed. For growth factor studies 70 mg of the lyophilised material was used whereas only 50 mg were required for steroid measurements.

Extraction of growth factors

Lyophilised samples were resuspended in 1 ml buffer (50 mmol l $^{-1}$ Tris, 5 mol l $^{-1}$ of phenyl-methylsulphonyl fluoride, 2 mmol l $^{-1}$ of EDTA pH 7.4) containing 1,000 counts of either ^{125}I -EGF or ^{125}I -TGF α to monitor manipulative losses. The samples were homogenised with a glass/glass Dounce Homogeniser and left on ice for 1 h to equilibrate. The homogenates were then centrifuged, the supernatants saved and the resultant pellets were resuspended in 2 ml of enzyme mixture (230 U ml $^{-1}$ of collagenase, 125 U ml $^{-1}$ of hyaluronidase in 150 mmol l $^{-1}$ of NaCl) and incubated overnight at 37°C ; these conditions were found to be optimal and ensured maximum growth factor recovery. The digested pellets were subsequently sonicated (six cycles of 20 s with 1 min cooling interval at an amplitude of 22μ ; MSE soniprep 150) the resultant mixture was centrifuged as before, the supernatant saved and the pellet was resuspended in 2 ml Tris buffer. Homogenisation, centrifugation and resuspension were then repeated twice with the supernatants always being saved. The supernatants were then pooled and the recoveries were assessed. The mean recovery of ^{125}I -EGF added at the beginning of the extraction procedure was $72 \pm 6\%$ whilst that of ^{125}I -TGF α was $69 \pm 8\%$. The supernatants were finally snap frozen, lyophilised to dryness and stored at -70° until required.

Radio-immunoassays for hEGF and hTGF α

The concentrations of immunoreactive hEGF and hTGF α in the lyophilised samples were determined by a liquid phase competitive RIA. Briefly, the lyophilised material was reconstituted in 700 μ l of PBS (0.02 mol l⁻¹ of Na₂HPO₄·2H₂O, 0.02 mol l⁻¹ Na₂HPO₄·2H₂O, 0.9% NaCl, 3.7 g l⁻¹ of EDTA, 1 g l⁻¹ of BSA, pH 7.4) and the assays were performed in triplicate employing 100 μ l of the reconstituted lyophilised material. The radio immunoassay for hEGF was as described previously (MacDonald *et al.*, 1990). No cross reaction was found with transforming growth factor- α , which exerts all of its biological effects via the EGF receptor, up to concentrations of 1 μ g ml⁻¹. Cross reactivity of this antiserum with a large variety of other substances has been tested by other investigators (Gregory *et al.*, 1979; Jaspas *et al.*, 1985). TGF α content was analysed using a commercial kit (Peninsula Laboratory Europe Ltd, St Helens, Merseyside) with rTGF1 as radioiodinated tracer and hTGF α as reference standard. Antisera to the rTGF1 was raised in rabbits and purchased from Peninsula Laboratory Europe Ltd. The rabbit anti-rat TGF1 antisera recognises both mouse and hTGF α but does not crossreact with either mouse or hEGF. Half maximal inhibition of binding of the ¹²⁵I-peptide to the antibody occurred at 140 pg/tube.

Measurement of androgens

Specific radioimmunoassays for testosterone and dihydrotestosterone (DHT) were carried out after a single separation on Gelman ITLC plates using an antiserum against dihydrotestosterone (Guildhay Antisera Ltd, Guildford, Surrey). The extraction of the androgens from the tissue, chromatographic separation, radioimmunoassay and reliability of the method have already been described in detail (Habib *et al.*, 1976; Houston *et al.*, 1985). Briefly 50 mg of the lyophilised powdered tissue extract was reconstituted in 2 ml of Tris HCl buffer containing 1,000 cpm radioactive steroids for each of the androgens to be assayed for recovery. Following equilibration, the steroids were extracted three times with diethylether and the pooled extracts were dried down, reconstituted in 50 μ l ethanol and separated on thin layer chromatography plates. The steroids were subsequently extracted, reconstituted in PBS buffer, recovery was assessed and the radioimmunoassay carried out.

Protein determination

Protein was estimated by the method of Bradford (1976) using bovine serum albumin as standard.

Data analysis

All analysis were performed in triplicate and the data has been presented as mean \pm standard error of the mean. Differences in growth factors and androgen concentrations were tested for statistical significance by student *t*-test. Correlation of the degree of association for any two parameters was determined by calculation of the correlation co-efficient (*r*): *r* values presenting a probability of <0.05 were considered to be statistically significant.

Results

Figure 1 demonstrates the relative concentrations of EGF and TGF α in tissue extracts obtained from patients with BPH (*n* = 19) and prostate cancer (*n* = 19). The mean \pm s.e.m. values (ng g⁻¹ protein) for EGF (195.61 \pm 19.94) and TGF α (92.57 \pm 7.60) in the BPH specimens were slightly but not significantly (*P* > 0.3) lower than those measured in the cancer tissue extracts (EGF = 235.60 \pm 24.45; TGF α = 100.73 \pm 15.47).

Additionally the EGF/TGF α ratios in both tissue types were found to be of the same order of magnitude (mean

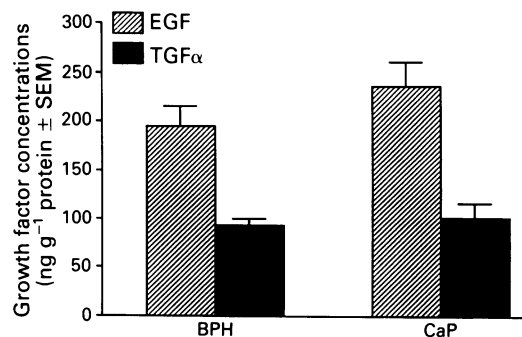


Figure 1 Comparison of immunoreactive EGF and TGF α in tissue extracts obtained from 19 patients with BPH and 19 patients with cancer of the prostate (CaP). The bars represent the mean values of all extracts within each group \pm s.e.m.

value for BPH = 2.12; CaP = 2.34) with the EGF concentration in each tissue extract being approximately twice the TGF α concentration.

Analysis of the individual EGF and TGF α values in BPH and CaP by-linear regression (Figures 2 and 3) resulted in a direct correlation between EGF and TGF α in both BPH (*r* = 0.72, *P* < 0.001) and CaP (*r* = 0.69, *P* < 0.001).

Furthermore when 13 of the tumours were classified according to their histological grade (Gleason score: primary + secondary pattern) and these were in turn correlated to their growth factor concentrations, a significant correlation was found between the Gleason score and EGF (*r* = 0.65, *P* < 0.02) and the Gleason score and TGF α (*r* = 0.57, *P* < 0.05) with the concentration of the growth factors in the tissue extracts increasing as the tumour becomes less differentiated (Table I).

Testosterone and DHT concentrations were also measured in the tissue extracts. These measurements confirmed our earlier findings revealing significantly higher levels of testosterone in CaP (289.26 \pm 76.9, *n* = 12) when compared to BPH (94.02 \pm 14.4, *n* = 19) whilst DHT concentrations were significantly higher in BPH (201.64 \pm 38.4) than in CaP tissue extracts (79.11 \pm 27.8). The differences between the two groups were statistically significant (*P* < 0.01).

Attempts to correlate the steroid hormone levels with the growth factor concentrations employing linear regression

Table I Effect of tumour differentiation on tissue EGF and TGF α concentrations

Gleason score	EGF (ng g ⁻¹ protein)	TGF α (ng g ⁻¹ protein)
2-4	135.08 ^a (1)	32.24 (1)
5-7	203.78 \pm 24.08 ^b (8)	91.62 \pm 12.95 (8)
8-10	359.18 \pm 54.06 (4)	123.38 \pm 41.6 (4)

^aParentheses denote number of tumours in each group. ^bMean \pm s.e.m.

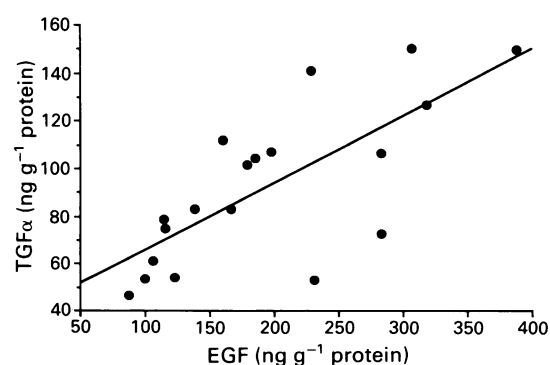


Figure 2 Relationship between the concentration of immunoreactive EGF and that of immunoreactive TGF α in prostate tissue extracts from 19 patients with benign prostatic hyperplasia (*r* = 0.72, *P* < 0.001).

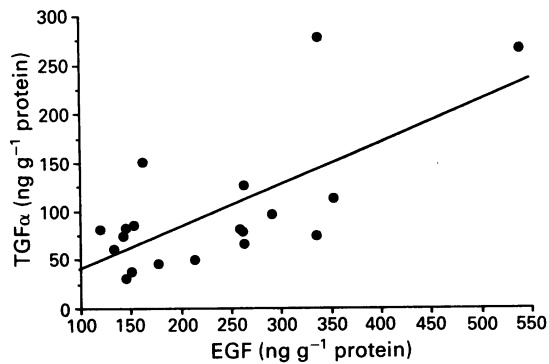


Figure 3 Relationship between the concentrations of immunoreactive EGF and TGF α in the tissue extracts obtained from 19 patients with carcinoma of the prostate ($r = 0.69$, $P < 0.001$).

yielded positive correlation only between testosterone and EGF ($r = 0.62$, $P < 0.05$) and testosterone and TGF α ($r = 0.61$, $P < 0.05$) in CaP (Figure 4). No similar correlation was found in BPH or indeed between DHT and the growth factors for both tissue extracts.

Discussion

The present study confirms the presence of immunoreactive EGF and TGF α in tissue extracts from hyperplastic and

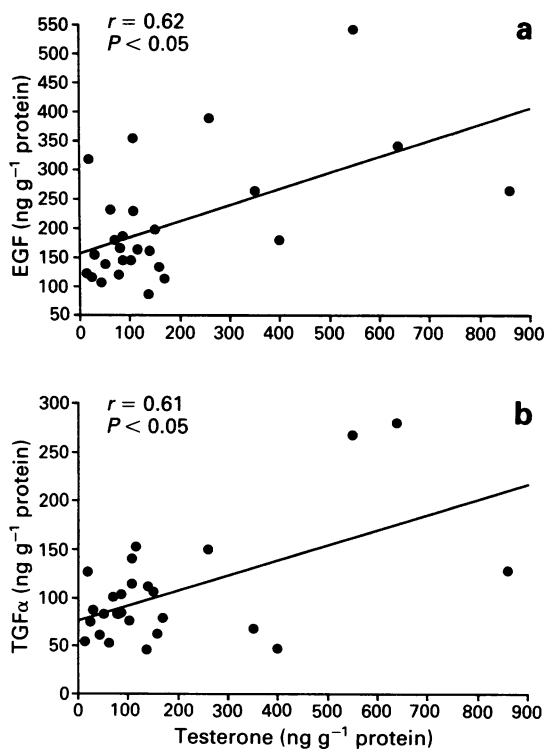


Figure 4 The correlation between the concentrations of testosterone and EGF **a**, and testosterone and TGF α **b**, as measured in tissue extracts obtained from 12 patients with cancer of the prostate. Regression lines are shown with correlation coefficients (r).

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- neoplastic human prostates. These findings are consistent with the reports on the production of EGF-related peptides in human prostate tissue (Gregory *et al.*, 1987; Fowler *et al.*, 1986) and TGF α -like molecules by prostate cancer cell lines (Connolly & Rose, 1989; MacDonald *et al.*, 1990; Hofer *et al.*, 1991). TGF α was originally characterised by its ability to induce phenotypic transformation of normal cells (Sporn & Roberts, 1985) and the elevated expression of this growth factor in transformed cells has been reported (Marquardt *et al.*, 1983). However, TGF α has since been found also in a variety of other normal as well as neoplastic tissues (Coffey *et al.*, 1987; Gomella *et al.*, 1989; Lloyd *et al.*, 1992), suggesting a possible role for this growth factor in normal cell physiology.
- The expression of TGF α in human benign prostate tissue has not previously been reported. In the current study, the TGF α concentrations in BPH were compatible with those measured in other tissues (Liu *et al.*, 1990). It was also of interest to note that there was no difference in the TGF α levels between BPH and prostate cancers even though there was a marginal increase in TGF α expression as the tumour became less differentiated. Significantly however, this increase in TGF α concentrations was manifested at a time when the expression of the EGF-receptor is down regulated (Maddy *et al.*, 1989) thus raising further the possibility that TGF α may be exerting its biological effects by interacting with receptor sites other than those associated with EGF (Winkler *et al.*, 1989). Although one should not exclude the possibility that higher levels of EGF-R activation can also result in more rapid receptor turnover and/or down regulation of receptor expression.
- Until recently TGF α was thought to be the counterpart of EGF in cancer, produced by the neoplastic prostate cell at the expense of EGF as part of an autocrine regulatory mechanism to reduce the need of these cells for exogenous mitogens (MacDonald *et al.*, 1990; Hofer *et al.*, 1991). However the presence of immunoreactive EGF in extracts from BPH and cancer at equivalent concentrations highlights, once more, the complexity of the biological actions of these two growth factors in the human prostate. Additionally, the close association found between TGF α and EGF in the two tissue types suggests that the mechanism responsible for the secretion of one of these growth factors may also be responsible for controlling the production of the other. Whether both growth factors act in synergy to induce neoplastic transformation in the human prostate still remains to be clarified. In view of the increasing interest in the role of growth factors in endocrine related tumours (Bates *et al.*, 1986; Lai *et al.*, 1989; MacDonald & Habib, 1992; Perheentupa *et al.*, 1984) attempts were also made to establish the relationship between steroid hormone concentrations and the growth factors in the prostate extracts. Whilst there was some correlation between testosterone and TGF α and EGF in prostate cancer, the absence of a parallel association in BPH where DHT is the active steroid may possibly suggest an indirect role for testosterone in the regulation of prostatic EGF and TGF α . Though this relationship will need to be confirmed in a more dynamic system, the production of growth factors in some tissues has been shown to be sensitive to sex hormones. This has been witnessed in a number of systems including the submaxillary gland where testosterone increases EGF levels (Perheentupa *et al.*, 1984), in various human cancer cell lines where oestradiol influences the production of EGF related polypeptides (Lippman *et al.*, 1986) and in breast cyst fluid where androgens modulate EGF concentrations (Lai *et al.*, 1989).

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