

LETTER TO THE EDITOR

METASTATIC LESIONS FROM PROSTATE CANCER DO NOT EXPRESS OESTROGEN AND PROGESTERONE RECEPTORS

Hobish *et al.*¹ have recently indicated that neither lymph node nor distant metastases from prostate cancer patients apparently express oestrogen receptors (ERs) and progesterone receptors (PRs), as revealed by immunohistochemical analysis. Incidentally, the authors report that different human prostate cancer cell lines (LNCaP, DU145, and PC3) were also found to be negative for both ERs and PRs using multiple experimental approaches, namely reverse transcriptase-polymerase chain reaction (RT-PCR), ligand binding assay (LBA), and immunocytochemical assay (ICA).

Although the absence of ERs or PRs from metastatic lesions of advanced human prostatic carcinoma could be in some respect predictable, all the above evidence apparently conflicts with our previous reports in which, using RT-PCR, LBA, and ICA approaches, LNCaP cells were found to express not negligible amounts of ER but also PR² and the ER-associated heat shock protein 27 kD (hsp27),³ which has been proposed to serve as a marker of oestrogen sensitivity in both breast and endometrial epithelial cells.⁴ On the contrary, PC3 cells displayed only nuclear ER, though in far lower amounts, and were PR-negative, as revealed by LBA and ICA.^{2,5}

Based on the fact that in *in vitro* systems, a heterogeneous cell line is invariably exposed to the numerous selective pressures of an artificial environment, it is hard to figure out the reasons for this apparent discrepancy. Furthermore, a number of methodological differences may also account for this inconsistency, but to embark here in a technical debate seems inappropriate and may eventually result in confusion.

There remain, however, significant unresolved issues concerning the mechanisms responsible for the susceptibility of prostate cancer tissues and cells to oestrogen action. As repeatedly reported by our own and other groups,^{2,6–9} growth of LNCaP cells is significantly stimulated by physiological concentrations (0.01–1 nM) of oestradiol (E₂). Furthermore, the growth increase induced by E₂ is even greater than that observed using either natural (DHT) or synthetic (R1881) androgens, under exactly the same experimental conditions. This effect is mediated through binding of oestradiol to genuine ER, being completely abolished by the pure anti-oestrogen, ICI-182,780, but unaffected by the pure anti-androgen, casodex.² Equally, we have observed that E₂ inhibits the growth of androgen non-responsive PC3 cells, presumably through a post-transcriptional increase of TGFβ.⁵

Earlier studies failed to detect ERs from nuclear extracts or cytosol fractions of LNCaP cells.^{7,10} These studies have put forward the hypothesis that the

E₂-induced increase of proliferative activity of LNCaP cells is due to oestrogen binding to a point-mutated form of androgen receptors (ARs),¹¹ having an increased affinity for progestagenic and oestrogenic steroids.¹² This hypothesis, however, is not fully convincing, as the relative binding affinity of E₂ for this abnormal form of AR has been reported to be much lower (0.4–6 per cent) than that of the synthetic androgen R1881¹³ and, therefore, could not accommodate the remarkable growth effects observed with E₂ in this cell line.

This is far from being the whole story. Oestrogens and their antagonists, anti-oestrogens, exert an array of different biological actions on prostate tumour cells, including induction of PSA,² up-regulation of the cell-cell adhesion molecule E-cadherin,¹⁴ and increase of cell death rates.¹⁵ A pointed example is given by the remarkable induction of apoptosis seen in LNCaP cells after exposure to the synthetic oestrogen diethylstilboestrol.¹⁶ Correspondingly, it has been emphasized that intravenous stilboestrol diphosphate may have a direct cytotoxic effect on prostate tumours, resulting in high response rates in patients having metastatic, androgen-refractory disease.¹⁷

Although it is conceivable that at least some of these effects may involve extrareceptor mechanisms (such as transcriptional activation at AP1 sites or modulation of intracellular cAMP levels), the concurrent evidence for both ER presence in prostate cancer tissues or cells and the removal of oestrogen effects by anti-oestrogens may well depict a case of classical response or sensitivity to hormonal stimulus.

Recently, evidence has accumulated for the existence of two distinct forms of ER, ERα and ERβ, with different tissue distribution and transactivation properties.^{18,19} The presence of ERβ in human ovaries and urogenital tract, including prostate, may explain how these tissues are sensitive to oestrogen action in the absence of the first identified ER, ERα.²⁰ This may help our understanding of the amazing array of oestrogen activities, often opposite, even at non-classical target tissue sites, and at least some paradoxical effects of oestrogen-like drugs.

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AUTHORS' REPLY

We read with interest the comments of Drs Castagnetta and Carruba regarding our manuscript¹ on expression of oestrogen (ER) and progesterone receptors (PR) in metastatic lesions from prostate cancer. We agree that some aspects of oestrogen action in the prostate are not completely understood. For example, there may be a role for the recently discovered ER- β in prostatic disease and this issue will probably be investigated in the future.

However, we feel that there is conclusive evidence that ER- α in human prostate is located in the stromal compartment and that epithelial cells, including tumour cell lines, do not express ER- α and PR.^{1–6} Not only our group, but also Sonnenschein *et al.* applied more than one methodological approach to determine whether cell lines derived from metastatic lesions from human prostate cancer express these receptors.^{1,5} The recently reported apoptosis-inducing effect of diethylstilboestrol (DES) on prostate cancer cell lines is independent of ER expression.⁷

We also infer that some points raised in Dr Castagnetta's and Dr Carruba's letter deserve a more detailed analysis. The observation by Castagnetta *et al.* that oestradiol stimulates proliferation of LNCaP cells is in agreement with observations made by others.^{8,9} Castagnetta *et al.* were unable to block this effect of oestradiol with the pure anti-androgen bicalutamide (Casodex) and concluded that oestradiol therefore does not stimulate proliferation only by interaction with the mutant androgen receptor (AR). Moreover, bicalutamide did not antagonize androgen and oestradiol-induced increase in prostate-specific antigen (PSA)

staining.⁹ However, in all these experiments the concentration of bicalutamide did not exceed 100 nM. Since the affinity of bicalutamide for AR is relatively low, in our experience a concentration of 100 nM is not sufficient to block AR activity. This view is confirmed by the experiments by Veldscholte *et al.*¹⁰ and Zhao *et al.*¹¹ Veldscholte *et al.* showed that higher concentrations of bicalutamide (1 μ M) were needed to inhibit the effect of synthetic androgen methyltrienolone on the growth of LNCaP cells.¹⁰ This concentration of bicalutamide was also necessary to antagonize the secretion of PSA protein into LNCaP supernatants.¹¹ These results clearly show that bicalutamide at concentrations of 1 μ M and higher is able to antagonize AR function in LNCaP cells and suggest that a cautious interpretation of the results of Castagnetta *et al.* is needed. Under their experimental conditions, one cannot exclude the possibility that the observed effects of oestradiol are mediated by the AR of LNCaP cells.

We also note that the role of the pure anti-oestrogen ICI 162 780 in LNCaP cells is not clear.^{9,12} It behaved as an inhibitor of oestradiol-induced proliferation in studies published by Dr Castagnetta's and Dr Carruba's laboratories. In contrast, its positive effect on E-cadherin expression was even more prominent than that of oestradiol. The interpretation of these results is not easy and it seems that this compound may display effects which are not related to the ER.

Finally, we would like to discuss the relationship between the increased binding affinity of oestradiol for the AR and the stimulation of proliferation. We agree that the binding affinity of oestradiol for the AR in