Short Communication

ENDOGENOUS PEROXIDASE: AN ALTERNATIVE TO OESTROGEN RECEPTOR IN THE MANAGEMENT OF BREAST CANCER?

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MANY WORKERS have shown that human breast cancers which contain oestrogen receptors (RE) are more likely to respond to hormonal therapy than those which do not (McGuire *et al.*, 1978). RE status, as determined by conventional techniques is a relatively poor indicator of responsiveness to hormonal therapies; only 35–63% of patients classified as RE⁺ respond to endocrine ablation (Roberts *et al.*, 1978; McGuire *et al.*, 1978). Standard RE assay techniques require up to 300 mg of tumour, an amount which is often unavailable in cases presenting early with small primary tumours, or where metastatic deposits are inaccessible to open biopsy (*e.g.* in bone or liver).

These limitations reduce the clinical usefulness of RE assays and have led to a search for alternative indicators of hormone responsiveness which might discriminate better and be detected in smaller tumour samples. Progesterone receptor (RP) (McGuire *et al.*, 1978) and endogenous peroxidase (Lyttle & De Sombre, 1977; Duffy & Duffy, 1977; De Sombre *et al.*, 1975) are two proteins which have been suggested as potential indicators of hormone responsiveness.

The present study was set up to assess the value of endogenous peroxidase as an indicator of hormone dependence by measuring peroxidase levels in rat mammary tumours which serve as models of hormone-dependent and -independent growth.

DMBA-induced rat mammary tumours are considered to serve as models of ovary-

dependent growth. Previous work in our department has shown that over 80% of such tumours regress after oophorectomy (Hawkins *et al.*, 1978; Scott *et al.*, 1979). Twenty-four such tumours were examined in this study. Two transplantable lines (TG3 and TG5) of rat mammary tumour which exhibit ovary-independent growth (Hawkins *et al.*, 1978) have been generated in our department and 20 tumours of these lines were also examined.

Each of the 44 tumours was dissected free from the host animal, after exsanguination. The tumour was then homogenized in an ice-cooled tube, at a concentration of 300 mg/ml in cold 10mM Tris buffer (pH 8.0) with 10% glycerol (v/v) using a Silverson homogenizer. The homogenates were centrifuged for 45 min at 39,000 *g* to yield a clear cytosol. A random sample of 12 cytosols from each group of tumours was then processed by mixing each with 100 μ l of monothio glycerol and assaying for RE and RP by saturation analysis techniques as described elsewhere (Hawkins *et al.*, 1977, and in preparation). The cytosols from the other tumours were discarded.

Each centrifugation pellet was then homogenized at 300 mg/ml in 10mM Tris buffer (pH 7.4) containing 0.5M calcium chloride to solubilize any peroxidase present. The homogenate was centrifuged for 45 min at 39,000 *g* to yield a clear cytosol containing the solubilized peroxidase. This extraction procedure is based on that of De Sombre & Lyttle (1978).

The cytosol was then assayed for per-

oxidase by a method based on that of Himmelhoch *et al.* (1969). The reaction mixture in the cuvette comprised 13mm guaiacol, 0.4mm hydrogen peroxide and 10mm Tris buffer (pH 7.4) containing 0.5mm calcium chloride, in a final volume of 3 ml. The reaction was started by addition of cytosol, a volume between 0.1 and 0.5 ml being required to give a suitable deflection of the spectrophotometer needle. The rate of reaction was measured by the change in absorbance at 470 nm between 1 and 3 min after starting the reaction.

Receptor concentrations were expressed in fmol/mg of wet tumour and peroxidase content in u/g wet tumour. One peroxidase unit was defined as the amount of enzyme required to produce an increase of one absorbance unit per minute under the assay conditions used.

FIG. 1.—Endogenous peroxidase content in rat mammary tumours which serve as models for hormone-dependent and independent growth. Each symbol represents one tumour.

The Wilcoxon rank-sum test was used to evaluate the differences between “hormone dependent” and “hormone-independent” tumours for concentrations of each “indicator-protein”.

Fig. 1 shows the endogenous peroxidase content of each of the 24 DMBA-induced tumours and each of the 20 transplantable tumours. Peroxidase was detectable in all but 2 of the DMBA-induced tumours, but was undetectable in 6 of the transplantable tumours. Analysis of the levels in the two groups revealed a highly significant difference ($P < 0.001$). Nevertheless there is a considerable overlap in peroxidase levels between the “hormone-dependent” and “independent” tumour models.

Figs. 2 and 3 show the levels of RE and RP in a random sample of 12 tumours from each group. Significant differences were again found between “hormone-dependent” and “independent” tumours. In the case of RE there was no overlap between the groups. RP was undetectable in one DMBA-induced tumour, but other-

FIG. 2.—Oestrogen receptor content in rat mammary tumours which serve as models for hormone-dependent and independent growth. Each symbol represents one tumour.

- peroxidases from the bone marrow of the guinea-pig. *Biochemistry*, **8**, 914.
- LYTTLE, R. C. & DE SOMBRE, E. R. (1977) Generality of oestrogen stimulation of peroxidase activity in growth responsive tissues. *Nature*, **268**, 337.
- LYTTLE, R. C., THORPE, S. M., DE SOMBRE, E. R. & DAEHNFELDT, J. L. (1979) Peroxidase activity and iodide uptake in hormone-responsive and hormone-independent GR mouse mammary tumours. *J. Natl Cancer Inst.*, **62**, 1031.
- MCGUIRE, W. L., HORWITZ, K. B., ZAVA, D. T., GAROLA, R. E. & CHAMNESS, G. C. Hormones in breast cancer, update 1978. *Metabolism*, **27**, 487.
- ROBERTS, M. M., RUBENS, R. D., KING, R. J. B. & 4 others (1978) Oestrogen receptors and the response to endocrine therapy in advanced breast cancer. *Br. J. Cancer*, **38**, 431.
- SCOTT, A. M., MURPHY, S. & HAWKINS, R. A. (1979) Synthesis of DNA and Lecithin in tissue culture and oestrogen receptor activity in rat mammary tumours dependent on and independent of the ovary. *J. Endocrinol.*, **81**, 183.