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. Progestogen 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. 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 μl of monothioglycerol 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 13 mM guaiacol, 0.4 mM hydrogen peroxide and 10 mM Tris buffer (pH 7.4) containing 0.5 mM 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.

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-
The results obtained in this study indicate that RE and RP assays clearly discriminate between hormone dependence and independence in these rat mammary tumours. It is known that not all DMBA-induced tumours are hormone-dependent and it might be postulated that the one such tumour in which no RP could be detected might have belonged to the hormone-independent minority of DMBA-induced tumours. This study revealed no overlap in RE levels between the two groups but it should be noted that in earlier generations of the TG3 and TG5 lines higher RE concentrations were found, and the distinction between the groups was less clear-cut (Hawkins et al., 1978; Scott et al., 1979).

Our results support the postulated relationship between peroxidase content and hormone dependence. However, the overlap in peroxidase levels between the largely hormone-dependent, DMBA-induced group and the hormone-independent transplantable group was considerable. It seems unlikely, therefore, that endogenous peroxidase will prove to be a more reliable discriminator than RE.

Our findings are somewhat at variance with those of Lyttle and his co-workers (1979) in their studies of peroxidase levels in mouse mammary-tumour models. They found no overlap between hormone-dependent and independent groups.

Peroxidase can be readily identified by a simple histochemical technique (De Sombre et al., 1975) and could, therefore, be detected in much smaller biopsy specimens than are needed for standard RE assays. Peroxidase estimations in breast tumours might, therefore, prove clinically useful in overcoming one of the limitations of standard receptor assays—namely, the amount of tumour needed. However, the findings of this study suggest that peroxidase is unlikely to provide a more accurate prediction of hormone-responsiveness than RE.

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