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Short Communication

HUMAN BREAST TUMOUR CYTOSOL OESTROGEN RECEPTOR BINDING TO OLIGO(dT)-CELLULOSE

L. MYATT, J. O. WHITE, M. D. FERNANDEZ* AND J. I. BURN†

*From the Institute of Obstetrics and Gynaecology, Hammersmith Hospital, London, W12 0HS the *Department of Chemical Pathology, and the †Department of Surgery, Charing Cross Hospital, London W6 8RF.*

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THE SUCCESSFUL PREDICTION of objective remission in breast tumours following hormonal therapy may be improved if the functioning of the oestrogen-receptor (RE) system is assessed by the presence of both cytosol and nuclear RE (RE_c and RE_N) (Garola & McGuire, 1977; Leake *et al.*, 1981), or both RE_c and progesterone receptor (RP) (Horwitz *et al.*, 1975; McGuire *et al.*, 1977; King *et al.*, 1979). However, there are certain practicalities that often preclude satisfactory receptor analyses, including insufficient tissue for saturation analysis and the instability of RP and RE_N under *in vitro* assay conditions. The regulation of RE_N binding in animal tissues may be controlled by a factor which activates RE_C, increasing its affinity for nuclear binding sites and transforming it to the RE_N (Thrower *et al.*, 1976; White *et al.*, 1978; Myatt *et al.*, 1982). The concentration of this putative activating factor is assessed by the binding of RE_C to artificial nuclear acceptor matrices including oligo-(dT)-cellulose and DNA-cellulose. Variations in the capability of breast-tumour RE_C to bind to DNA-cellulose, as described by Sato *et al.* (1981), may influence the extent of RE_N binding. Breast tumours that contain RE_C which cannot be converted to RE_N exhibit a poor objective response to endocrine therapy (Kute *et al.*, 1978). In the present study the concentration of RE_c and its ability to bind to oligo(dT)-cellulose has been measured and correlated with the presence of RE_N and RP_C. This may evaluate whether the presence of an activated RE (*i.e.* capable of binding to an artificial nuclear matrix) is a determinant of a functionally responsive receptor system in human breast tumours.

Sixty-three biopsy specimens from 61 patients (22–79 years of age) with histologically confirmed primary breast carcinoma were studied. Thirty-eight patients (62·3%) were postmenopausal and the remaining 23 (37·7%) premenopausal. Tumour tissue was stored in liquid N_2 for not more than 2 months. Tissues were pulverized in a microdismembrator (Braun Instruments Ltd, West Germany) and resuspended in buffer (10 mM phosphate; 1·5 mM diK EDTA; 10 mM monothioglycerol (pH 7·4) containing 30% glycerol) to a concentration of 1:8 (w/v). Subsequent procedures were performed at 4°C. The suspension was stirred for 10 min, followed by centrifugation at 2000 *g* for 15 min to yield a nuclear pellet and supernatant (cytosol) fraction. RE_C and RP were assayed using the method of King *et al.* (1979) in 200 μl aliquots of the supernatant at 4°C overnight. RE_C was measured using 5 nM (^{3}H)-oestradiol with 1 μM diethylstilboestrol as competitor, and RP using 10 nM(^{3}H)-progesterone plus 1 μM cortisol with 1 μM norethisterone as competitor. Labelled RE_C complexes were chromatographed on columns containing 100 mg oligo(dT)-cellulose (Myatt *et al.*,

TABLE I.—*Breast-tumour sex-steroid receptor concentrations*

	Premenopausal					Postmenopausal		
	Mean ± s.e. (fmol/mg wet wt)	P*	% +ve	P†	n	Mean ± s.e. (fmol/mg wet wt)	% +ve	n
RE_C	0·60 ± 0·22	< 0·01	69·6	—	23	2·82 ± 0·60	77·5	40
dT‡	0·13 ± 0·04	< 0·05	29·4	0·023	17	0·47 ± 0·12	64·7	34
RE_{N30}	0·36 ± 0·16	—	55·0	—	21	0·49 ± 0·10	65·8	38
RE_{N4}	0·27 ± 0·05	< 0·1	75·0	—	21	0·56 ± 0·10	71·1	38
RP_C	0·25 ± 0·06	—	56·5	0·046	23	0·19 ± 0·08	31·6	38

* For difference in concentration from postmenopausal using t test
† For difference in incidence from postmenopausal using Fisher's exact test
‡ Binding to oligo(dT)-cellulose

1978). The crude nuclear pellets were resuspended and washed ×3 in TEDG buffer (10 mM Tris/HCl; 1·5 mM EDTA; 1 mM dithiothreitol containing 10% glycerol, pH 7·6) before resuspension in 9 volumes (w/v) of buffer. Aliquots (200 μl) of this suspension were incubated with 10 nM (^{3}H)-oestradiol with 2 μM diethylstilboestrol as competitor, for 1 h at 30°C (total receptor) and 4°C (available receptor). Nuclei were then washed ×3 with 1 ml of 1% BSA in TEDG buffer, followed by a final wash with 1 ml TEDG, after which radioactivity was extracted with 2×2 ml ethanol and counted. A receptor concentration of >0·1 fmol/mg wet tissue was taken as positive, for all parameters.

In the postmenopausal group the concentration of RE_C was significantly increased, in agreement with other findings (Hawkins *et al.*, 1980), as was its capacity to bind to oligo(dT)-cellulose (Table I). The control of receptor content and its ability to bind to oligo(dT)-cellulose (by interacting with activating factor to produce the 5S form of the receptor (Myatt *et al.*, 1982)) are apparently regulated quantitatively in a similar manner. There was no significant difference in concentration of RP_C between the 2 groups, in agreement with others (Barnes *et al.*, 1979; Lippman, 1980). The content of RE_N (30°C) was not significantly different in the 2 groups, but in the postmenopausal group the concentration of available RE_N (4°C) was significantly increased. The ratios of both RE_{N30} and RE_{N4} to RP_C were higher in the postmenopausal group (RE_{N30}/RP_C: 1·31 ± 0·26 (n = 7) pre- *vs.* 4·44 ± 1·16 (n = 13) post-, $P < 0·02$; RE_{N4}/RP_C: 1·46 ± 0·50 (n = 11) pre- *vs.* 4·38 ± 1·14 (n = 13) post-, $P < 0·05$).

There was no significant difference in the incidence of RE_C or RE_N (30°C and 4°C) between the 2 groups. The incidence of RP^+ tumours was significantly lower in the postmenopausal group, whilst the incidence of RE binding to oligo(dT)-cellulose was significantly higher. The absence of changes in total nuclear receptor (RE_{N30}) despite the postmenopausal increase in RE_C and plasma oestrogen may be due to the predominance of oestrone, which has a relatively low affinity for RE, in the plasma of postmenopausal women (Siiteri & MacDonald, 1973).

The presence of RE_{N4} in human breast tumours has been previously reported

TABLE II.—*Frequency of steroid-receptor combinations in breast tumours*

	RE_C	RE_{N30}	RP_c (No. patients) +	−
Premenopausal	+	+	7	0
	+	−	3	4
Postmenopausal	+	+	8	13
	+	−	1	6
		RE_{N4}		
Premenopausal	+	+	7	3
	+	−	3	1
Postmenopausal	+	+	8	16
	+	−	1	3
		dT		
Premenopausal	+	+	5	0
	+	−	1	4
Postmenopausal	+	+	12	9
	+	−	0	6

(Garola & McGuire, 1977; Kiang, 1977, Laing *et al.*, 1977). The increase in the proportion of RE_{N4} that occurs in the postmenopausal group is in agreement with Thorsen (1979) and may be explained by the nature of the ligand that occupies the RE_N. As oestrogens may be formed by breast-tumour tissue *in situ* (Miller & Forest, 1974; Adams & Li, 1975; Li *et al.*, 1976) from precursors in plasma, a simplistic interpretation of the origin of RE_N based upon the nature of the circulating oestrogen may not be entirely valid. However, the change in content and proportion of RE_{N4} in pre- and postmenopausal groups suggests that they are not simply a constitutive form of nuclear receptor.

The low incidence of RP^+ tumours in postmenopausal women may be related to the value of receptor content used in discriminating between tumours or, alternatively, to insufficient oestrogen stimulation in the postmenopausal patient (McGuire, 1980). A positive correlation exists between RE content and response to endocrine therapy (McGuire, 1980; Lippman, 1980) with postmenopausal patients showing the most favourable response (Leake *et al.*, 1981). A better index of function may be the ratio RP/RE_N, as proposed for the assessment of human endometrium (King & Whitehead, 1980).

The rationale that each parameter represents a functional component of the receptor system has been verified by the improved prediction rate for response to hormone therapy when receptor parameters were measured simultaneously. In our group of premenopausal patients (Table II) selecting RE_C^+ and RE_{N30}^+, or RE_C^+ and RE_{N4}^+, was associated with being RP^+ (7/7 and 7/10 respectively). All tumours that were RE_C^+ and positive for binding to oligo(dT)-cellulose were also RP^+ (5/5). Binding to oligo(dT)-cellulose is therefore as good a parameter as RE_N content in predicting the presence of RP and hence response to hormone therapy.

In the postmenopausal RE_C^+ tumours there was little correlation between binding to oligo(dT)-cellulose or RE_N content and RP positivity. In contrast to the 78% of RE_C^+ tumours which were positive for binding to oligo(dT)-cellulose, only 37% were RP^+. In view of the apparent association between RE content and response to therapy, the postmenopausal group would be expected to have the better prognosis. However, on the basis of the simultaneous incidence of RE_C and RP, the postmenopausal group (37%) appears less favourable than the premenopausal group (68%). Binding to oligo(dT)-cellulose in RE^+ tumours (50% pre-, 78% postmenopausal) may be a better determinant of receptor function, and hence response to hormone therapy.

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