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ALKALINE PHOSPHATASES AND STEROID RECEPTORS IN HUMAN BREAST CANCER

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Summary—Estrogen receptors (ER), progesterone receptors (PR) and alkaline phosphatases (AP) were measured in 150 tumors from patients who underwent mastectomy for primary breast cancer. The percentage of ER positive samples was inversely related to the AP activity ranging from 88.9% in low activity samples (<30 U/mg prot.) down to 30.6% in the high activity ones (>400 U/mg prot.). When considering only ER positive samples, the ER content was inversely related to the AP activity. This could not be demonstrated for PR. Therefore, the authors suggest the hypothesis that in human breast cancer, the AP may play a role in the dephosphorylation of the ER molecule and in the consequent modulation of its binding capability.

INTRODUCTION

Although the presence of estrogen receptors (ER) and progesterone receptors (PR) is a well-established marker of breast cancer hormone sensitivity, a significant number of tumors behave in apparent disagreement to the receptor status. As a matter of fact, no more than 70–80% of patients displaying positivity for both ER and PR was reported to benefit from endocrine therapy, whereas a response can be obtained in 10–15% of ER negative–PR negative patients, and in 15% of the ER positive–PR negative ones [1, 2]. Moreover, the possible prognostic role of steroid receptors as a marker of the disease free interval or overall survival is still controversial [3].

The mechanism by which the tumor cell is controlled by the estrogens is very complex and the presence of steroid receptors must be considered necessary but not sufficient to ensure the tumor hormone sensitivity.

The ER phosphorylation has been reported to be essential in the calf uterus and in the chick oviduct to accomplish the high affinity binding to the ligand [4, 5]. In both cases the activation (phosphorylation) has been proved to be reversible and the binding capability of the inactivated estrogen receptors could be restored by *in vitro* incubation with ATP and ADP in the presence of metal ions [6].

The activating phosphorylation was showed to take place on the tyrosine residues of the receptor molecule and to be catalyzed by the tyrosine kinase enzyme [7]. Those proteins which are phosphorylated on tyrosine are the preferential substrate of alkaline phosphatases. This group of membrane-bound glycoproteins has been proposed to be involved in the regulation of the phosphorylation–dephosphorylation mechanism of the proteins which are phosphorylated at tyrosine residues [8].

Following the indications of such reports, we studied the possible relationships between the alkaline phosphatases (AP) and the estrogen binding activity in the human breast tumor tissue.

PATIENTS AND METHODS

150 samples were obtained from patients who had undergone mastectomy for primary breast cancer.

Tissue homogenization and cytosol preparation were carried out following the recommendations of the EORTC [9]. Steroid receptors were assayed by the conventional binding technique and total proteins by Coomassie blue staining as already reported [10] samples containing more than 10 fmol/mg protein being considered as positive. AP activity was measured on an aliquot of the same cytosol by spectrophotometry at 37°C according to the recommendations of the German Society of

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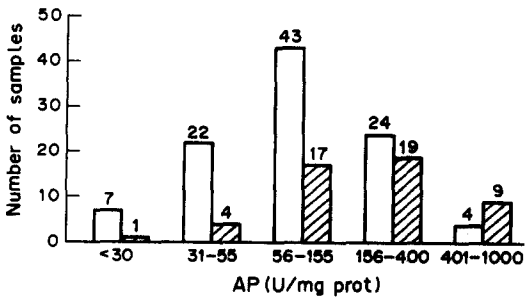

Fig. 1. Distribution of log transformed values of the AP activity in 150 breast tumor samples. The numbers above the columns refer to the number of ER positive (white columns) and ER negative (hatched columns) tumors.

Clinical Chemistry [11] by using 4-nitro-phenyl-phosphate as the substrate and diethanolamine as the assay buffer.

RESULTS

The AP activity was detectable in all the examined tumor samples ranging from 18 up to 924 U/mg prot. (median 119). The enzyme value was not affected by either the tumor size or the nodal status of the patient (Kruskal–Wallis test).

The receptor content of the tumors, in terms of both the percentage of positive samples and of absolute concentration was in agreement with most of the reported data.

The overall distribution of the steroid receptors was: ER+Pr+: 57.1%, ER+PR−: 9.5%, ER−PR−: 21.8% and ER−PR+: 11.6%. It resulted that the proportion of positive ER decreased significantly (χ^2-test, $P < 0.01$) on increasing the AP activity (Fig. 1). On the contrary the same behavior could not be demonstrated in the case of the PR.

When considering only the ER positive samples, a highly significant ($r = -0.635$, $P < 0.001$, $n = 102$) inverse correlation was found between the AP activity and the ER concentration.

A higher AP activity in the ER+ samples with respect to those of the ER-ones could be confirmed both in pre- and post-menopausal samples, but in the latter situation the statistical significance appeared to be more evident (Table 1).

Table 1. Tumor tissue AP activity (U/mg prot.) as a function of menopausal status and ER

		Pre-menopausal			Post-menopausal		
Samples	*n*	Median	Range	*n*	Median	Range	*P*[a]
All	63	145	16–738	87	84	16–924	<0.01
ER+	36	122	16–454	66	69	16–336	<0.01
ER−	27	163	18–738	21	181	33–924	NS
P[b]		<0.05			<0.01		

[a] Wilcoxon test, pre- vs post-menopausal samples.
[b] Wilcoxon test, ER+ vs ER− samples.
NS = not significant.

Table 2. Tumor tissue AP activity (U/mg prot.) as a function of both ER and PR presence

AP	ER+PR+	ER+PR−	ER−PR+	ER−PR−
Median	79	93	169	175
Range	18–454	21–231	63–535	18–924
n	84	14	17	32

ER+ groups results significantly different from the ER− ones irrespective of the PR presence ($P < 0.001$, analysis of variance).

Pre-menopausasl samples contained higher AP activity, but only in ER+ tumors (Table 1).

Finally, when considering the AP activity as a function of both the presence of ER and PR, only ER accounted for the difference of the enzyme activity, irrespective of the presence of PR (Table 2).

DISCUSSION

In our study, we could confirm the presence of a *p*-nitro-phenylphosphate phosphatase activity in the cytosol from breast cancer tissue. Such an activity, which has been reported to reside on the same protein of the phosphotyrosine-phosphatase [8] was inversely related to both the presence and the concentration of ER as measured by the conventional binding test. In contrast, PR do not seem to be similarly affected by the enzyme activity.

As a further confirmation of the AP/ER relationship, the post menopausal samples contained lower enzyme activity than the pre-menopausal ones, in agreement with the well known higher ER content of post-menopausal samples. As expected, AP values were high in ER-samples, regardless of the menopausal status.

Other authors have demonstrated, in calf uterus, that the estradiol receptor is phosphorylated on tyrosine and the phosphorylation is required for hormone binding of the receptor. The same authors have reported that the binding capability can be reversed by the action of purified phosphatase [6, 7].

Even if our data does not provide any direct proof of the connection between alkaline-phosphatase and receptor phosphorylation, the results of the above mentioned authors may provide a possible explanation of the relationship between AP and ER. If this were true, it would result that the cell hormone sensitivity may be modulated also at post-translational level by a reversible phosphorylating mechanism.

In conclusion, the measurement of some enzymatic activities which are related to the estrogen receptor content may be helpful in order to study the complex mechanism by which a breast tumor cell can be controlled by steroid hormones.

Moreover, the follow-up of the patients whose tumors have been characterized in the present study will elucidate if the measurement of the AP activity in addition to the steroid receptor may be of significance in selecting those patients who could benefit from endocrine therapy.

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