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Association of Plasminogen Activator Activity and Steroid Receptors in Human Breast Cancer

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Abstract—*Plasminogen activating activity (PAA) has been determined in 105 human breast cancer cytosols with a colorimetric assay. PAA has been related to steroid receptors in the same cytosols. A strong positive correlation between PAA and progesterone receptor levels was found (Spearmans coefficient of rank correlation, R = 0.74, P < 0.001). PAA discriminates between progestrone receptor-positive and -negative tumours, irrespective of oestradiol receptor content. It is suggested that PAA is a marker of functional oestradiol receptor and that fibrinolytic activity may be a causative factor in the low metastatic potential of progesterone receptor-positive tumours.*

INTRODUCTION

MANY transformed cells and cancers of animal and human origin produce plasminogen activators [1–3], a group of proteases which convert plasminogen into the active fibrinolytic enzyme plasmin. Plasminogen activator activity (PAA) has been reported to be a marker of neoplastic transformation [4–6]. PAA have been demonstrated in both normal and transformed rodent and human breast cells [1, 2, 7]. The expression of PAA is hormonally regulated in the Shionogi SC 115 murine carcinoma [8] and in the human breast cancer cell line MCF-7 [9].

The presence of PAA in solid human breast tumours has been related to receptor cleavage [10] and to hormone responsiveness [11]. PAA is usually measured indirectly through the lysis of radioiodinated fibrin. In the present paper, the chromogenic substrate S-2251 (H-D-Val-Leu-Lys-*p*-nitroanidide) has been used to assay PAA in cytosols from human mammary cancers. This method is reported to be more sensitive and reproducible than the fibrinolysis assay [12]. Results have been related to oestradiol and progesterone receptors in the same cytosols.

MATERIALS AND METHODS

Materials

Tumour tissue was obtained at mastectomy, immediately placed in liquid nitrogen and transported to the laboratory. Samples were stored at −70°C in a Revco Freezer (Revco Inc. W. Columbia, SC) until analyzed. Maximal storage time was 3 weeks.

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DCC assay

DCC assay of oestradiol and progesterone receptors was carried out with a single point saturation assay [13]. Cytosol was prepared by homogenization of tissue in 5 vol. of phosphate buffer (5 mM sodium phosphate, pH 7.4, 1 mM monothioglycerol, 10% glycerol) and centrifugation at 105,000 *g* for 60 min. Cytosol (200 μl) was incubated in duplicate with a final concentration of 5 nM [^{3}H]-steroid (oestradiol-17β or promegestone) with and without a hundred-fold excess of non-radioactive hormone. After 16 hours at 4°C, 500 μl of dextran-coated charcoal suspension was added (0.25% activated charcoal Norit A and 0.0025% dextran in 10 mM Tris/HCl, pH 8) and incubated for 15 min with shaking at 4°C. After centrifugation at 2000 *g* for 15 min 500 μl of the supernatant was counted.

Assay of PAA

PAA was measured essentially according to Overwien *et al.* [12]. Cytosol (50 μl) was diluted with 750 μl of Tris/HCl buffer (50 mM, pH 7.4), and 150 μl of freshly thawed human plasminogen solution (Kabi A/B, Stockholm, Sweden), 0.04 CU/ml, and 50 μl of chromogenic substrate (2.1 mg/ml of S-2251, Kabi A/B, Stockholm, Sweden) was added. The reaction mixture was thoroughly mixed and incubated for 120 min at 37°C. The reaction was stopped

by addition of 100 μl of 50% acetic acid. Control incubations containing all ingredients except plasminogen were used to determine plasminogen-independent proteolytic activity. A standard series of streptokinase solutions (15–120 IU/ml) were carried through the same procedure and used as a basis for calculation of PAA in the unknown samples. Optical density was read at 405 nm and converted to streptokinase units. PAA in the samples were taken as the difference between total and plasminogen-independent proteolytic activity.

RESULTS

Optical density at 405 nm was found to be linearly related to streptokinase activity in the concentration range 15–120 IU/ml. From Fig. 1 it is seen that cytosols gave parallel dilution curves in the same range of PAA values.

In most cytosols plasminogen-independent proteolytic activity was present and showed a negative correlation to progesterone receptor concentrations which, however, was barely significant (Spearmans coefficient of rank correlation $R = -0.18$, $0.025 < P < 0.05$).

Plasminogen-dependent proteolytic activity (PAA) was significantly correlated to both oestradiol and progesterone receptor concentrations. The Spearman coefficient of rank correlation was found to be 0.61 and 0.74 respectively, which is highly significant ($P < 0.001$). In Fig. 2 the relation between progesterone receptor and PAA is presented. A significant correlation between oestradiol and progesterone receptor levels was also demonstrated (Spearmans coefficient of rank correlation $R =$ 0.53, $P < 0.01$). When tumours were divided into four subgroups according to receptor content: ER + PR+, ER + PR−, ER − PR + and ER − PR−, PAA concentrations discriminated sharply between these guoups. Significantly higher levels of PAA were found in progesterone receptor-positive tumours, irrespective of oestradiol receptor content (Willcoxon's test for two samples, $P < 0.01$) (Fig. 3).

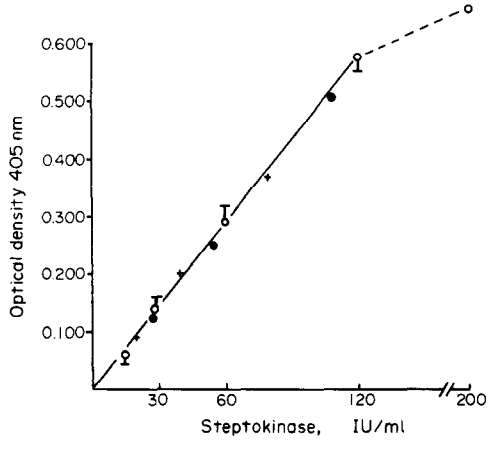

Fig. 1. Relation of optical density at 405 nm to streptokinase activity (IU/ml). Fifty microliter samples of streptokinase solution (15–200 IU/ml) were measured in duplicate (open circles). Solid circles and crossed line: optical density readings of serial cytosol dilutions. Solid bars: standard error of the mean (S.E.M.) of five different standard assays.

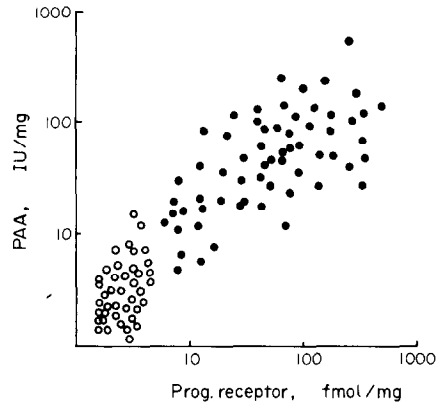

Fig. 2. Relation between plasminogen activator activity (PAA) and progesterone receptor levels. Solid circles: progesterone receptor-positive cytosols (> 5 fmol/mg protein). Open circles: progesterone receptor-negative cytosols. The Spearman coefficient of rank correlation: R = 0.74 (P < 0.001).

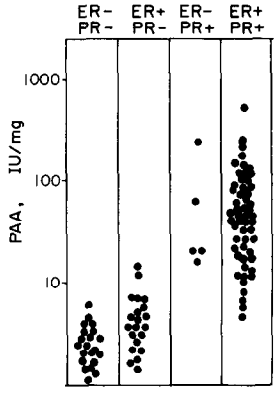

Fig. 3. Plasminogen activator activity (PAA) in four subgroups of tumours, classified according to their content of oestradiol and progesterone receptors: ER–PR–, ER + PR–, ER–PR + and ER + PR + . PAA was significantly higher in progesterone receptor-positive tumours compared to progesterone receptor-negative tumours, irrespective of oestradiol receptor content (Willcoxon's test for two samples, P > 0.01).

DISCUSSION

Plasminogen-independent proteolytic activity was present in a high percentage of cytosols. This would indicate that plasmin-like proteolytic enzymes are present in these

tumours, or that the cytosols contain varying amounts of endogeneous plasminogen which can be activated by PAA. A weak negative correlation between progesterone receptor levels and plasminogen-independent proteolytic activity was found. Cytosols with low or non-significant progesterone receptor values often had high concentrations of protease activity. These results may support the suggestion by Sherman *et al.* [10] that receptor cleavage is associated with plasmin-like proteases.

A highly significant positive correlation has been demonstrated between PAA and receptor content in breast tumour cytosols. Using a different method, these results corroborate the data presented by Sutherland [11]. PAA correlates better with progesterone than with oestradiol binding and discriminates between progesterone receptor-positive and negative tumours, irrespective of oestradiol receptor content. In the MCF-7 human breast cancer cell line PAA is induced by oestradiol [9], and the results presented would be compatible with the possibility that PAA, like progesterone receptor, is a marker of functional oestrogen receptors in this tissue [13, 14].

A large volume of experimental work emphasizes the importance of the coagulation system for the host/tumour relationship, tumour invasion and metastasis formation. When circulating intravascular cancer cells are prevented from forming a fibrinogen/fibrin network they seem to lose their ability to form metastases [15–19]. Fibrin depositions in tumours have been reported, and it has been suggested that such deposits form a necessary scaffolding that attracts new blood vessels to the area [20, 21]. Agents promoting fibrinolysis or blocking thrombus formation decrease the tendency to form metastases in animals injected with cancer cells, while antifibrinolytic agents like epsilonaminocaproic acid or trasylol enhance metastasis formation [18, 19, 22–24]. It seems possible that cells that are able to secrete plasminogen-activating substances might influence fibrin deposition in the vicinity of the cell and thus modify tumour growth and metastatic potential. Recently, Pichon *et al.* [25] reported a very low frequency of remote metastases in progesterone receptor-positive tumours which was inversely related to progesterone receptor concentration. The close correlation between PAA and progesterone receptor in human breast tumour cytosol would seem to indicate a causal relationship between fibrinolytic activity and metastatic potential in these tumours.

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