

Membranal tyrosine protein kinase activity (but not cAMP-dependent protein kinase activity) is associated with growth of rat mammary tumors

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DMBA induced rat mammary tumors were used to study the association of tyrosine protein kinase activity with tumor growth. Pharmacological manipulations of blood prolactin level, by perphenazine and bromocriptine, were used to stimulate or arrest tumor growth, respectively. During perphenazine treatment, a 2–3-fold increase in membranal tyrosine protein kinase activity, measured with angiotensin II as substrate, preceded the 3–4-fold increase in tumor area. At the same time the cAMP-dependent protein kinase activity, measured with kemptide as substrate, did not change.

Tyrosine protein kinase Mammary tumor DMBA Tumor growth

1. INTRODUCTION

Tyrosine protein kinases are enzymes that phosphorylate tyrosine residues of protein substrate. These protein kinase activities have been found in association with retrovirus transforming proteins [1,2]. Moreover, a number of polypeptide growth factor receptors including those for epidermal growth factor [3], platelet-derived growth factor [4], insulin [5] and somatomedin C [6] demonstrate tyrosine protein kinase activity. These results suggest a relationship between tyrosine protein kinase activity and the control of cell proliferation, although the exact nature of such a relationship is unknown. Less information is available on the role of tyrosine protein kinase in tissue growth. In a recent report, tyrosine protein kinase activity was shown to increase during embryogenesis [7]. The DMBA-induced mammary tumor may serve as an excellent model for such studies, as its growth rate is easily manipulated by hormones. Estrogens [8] and prolactin [9]

stimulate its growth, while ovariectomy [8], antiestrogens [10] and drugs which decrease plasma prolactin level cause growth arrest or regression of the tumors [11]. In the DMBA-induced mammary tumor as well as in the mammary gland and uterine tissue, changes in growth rate induced by hormones were associated with parallel changes in cAMP-independent quercetin-inhibited protein kinase activity [11–14]. We have recently shown the presence of tyrosine protein kinase activity in the membranes of these tumors [15]. This activity was inhibited by quercetin, similar to the cAMP-independent protein kinase activity that we measured previously in normal and malignant rat mammary tissues. In this report we describe the association between the membranal tyrosine protein kinase activity and the growth process induced by prolactin in these tumors.

2. MATERIALS AND METHODS

[³²P]ATP was obtained from Nuclear Research Center (Negev, Israel). DMBA, quercetin, cAMP, theophylline, angiotensin II and kemptide from

Abbreviation: DMBA, 7,12-dimethylbenz(a)anthracene

Sigma. Perphenazine from Schering Corp. (Kenilworth, NJ). Bromocryptine was a generous gift by Dr E. Flukiger, Sandoz (Basel, Switzerland).

2.1. Induction of tumors and preparation of membranes

Rat mammary tumors were induced by DMBA and their area was measured as described earlier [14]. Tumor growth arrest was achieved by daily injection of 0.5 mg bromocryptine for 10–14 days. Rapid tumor growth was then induced by daily injection of 1.0 mg perphenazine which causes an elevation of the plasma prolactin (Levy et al., submitted). The tumors were removed, frozen and crude membrane fractions were prepared as described [13]. The membranes were dispersed in the homogenization buffer containing 0.5% Triton X-100 and were used for protein kinase assays.

2.2. Protein kinase activity assays

Tyrosine kinase activity was measured by using angiotensin II as the peptide substrate as described previously [15,16]. cAMP-dependent protein kinase activity was measured using kemptide as the peptide substrate essentially as described by Kemp [17]. This activity was calculated by subtracting the phosphorylation obtained in the presence of cAMP from that obtained in its absence.

3. RESULTS AND DISCUSSION

The effects of cAMP and quercetin on the phosphorylation of both peptide substrates by the tumor membranes were checked (fig.1). Cyclic AMP increased the phosphorylation of kemptide threefold while quercetin had no effect on this activity (fig.1a). In contrast, the phosphorylation of angiotensin II was not affected by cAMP but was inhibited by quercetin, in the presence or absence of cAMP (fig.1b). The differential effects of cAMP and quercetin on the two activities indicate that the cAMP-dependent protein kinase does not interfere with the measurement of the tyrosine protein kinase or vice versa.

The two membranal protein kinase activities were measured during growth arrest (bromocryptine treatment), or on different days of tumor growth induction by perphenazine (fig.2). An almost threefold increase in tyrosine protein kinase

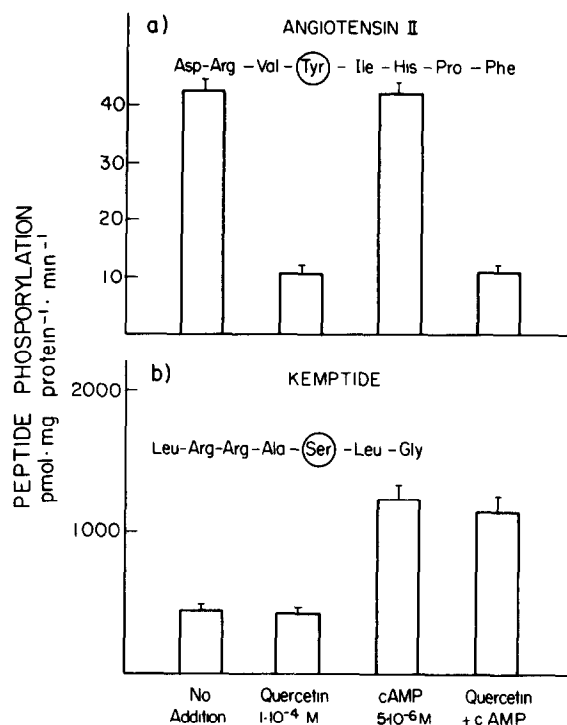


Fig.1. The effect of quercetin and cAMP on angiotensin II and kemptide phosphorylation. Peptide phosphorylation was measured as described in section 2.2. Quercetin, cAMP or both were added at the indicated concentrations. (a) Tyrosine protein kinase activity. (b) cAMP-dependent protein kinase. The results are the mean \pm SE of 3 experiments.

activity was evident immediately after the first day of perphenazine treatment, and the activity remained at the higher level during the five days of the treatment. At the same time the cAMP-dependent protein kinase activity did not change significantly. The tumor area changed at a slower rate, and an increase of two- to three-fold, that was significant by one-way ANOVA, was evident only after four days. These results indicate that activation of tyrosine protein kinases in tumor membranes, but not cAMP-dependent protein kinase, is an early event in the hormone-induced growth process and suggest that the enzyme activity may lead to the growth. The tyrosine protein kinase activity probably represents an important part of the cAMP-independent and quercetin-inhibited protein kinase activity which we previously found to

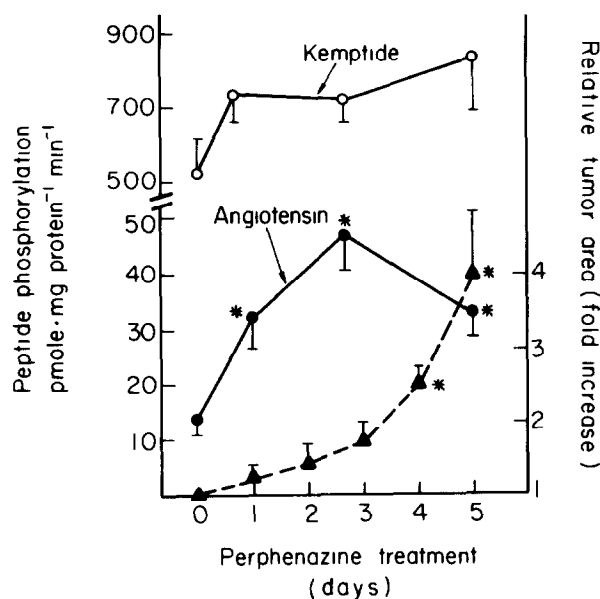


Fig.2. Stimulation of tumor growth, tyrosine protein kinase activity and cAMP-dependent protein kinase activity during perphenazine treatment. Tumor area at the end of bromocriptine treatment (day 0) was defined as -1 (relative area) and was followed during the next days of perphenazine treatment (\blacktriangle --- \blacktriangle). The two protein kinase activities were measured as described in section 2.2. (\bullet — \bullet) Tyrosine protein kinase activity; (\circ — \circ) cAMP-dependent protein kinase activity. The results are the mean \pm SE of 4–8 tumors. * denotes significant difference from day 0 ($p < 0.05$ by one-way ANOVA and Newman Keuls procedure).

be associated with growth of the rat mammary tumors ([14] and Levy et al., submitted).

Tyrosine kinase activity is associated with mitogens and growth factor receptors [3–6]. The presence of insulin [18] and EGF [19] receptors in the membrane of mammary tumors has been reported previously. Other tyrosine protein kinases such as some oncogenes, may also be located in the mammary tumor membrane [20,21] and thereby contribute to the enzymatic activity measured in this study.

Although the involvement of tyrosine protein kinase activity in the growth process was suggested quite some time ago, there are surprisingly few reports correlating the two phenomena *in vivo*. The activity of tyrosine protein kinase in the sea urchin embryo was markedly increased during early embryonic development [7]. Similar effects were

found in psoriatic skin. The increase in specific activity of tyrosine kinase in the psoriatic plaque corresponded well with an increase in mitotic index in this tissue as compared to normal skin [22]. On the other hand tyrosine protein kinase was found in a variety of tissues, some of which are known to grow slowly or not at all [23,24]. For example, higher tyrosine protein kinase activity in resting lymphocytes than in proliferating normal or leukemic blood cells was reported recently [23]. High tyrosine kinase activity was also shown in the particulate fraction from non-proliferating anuclear cells, such as platelets and red blood cells [24]. To the best of our knowledge this is the first report relating tyrosine kinase activity to the *in vivo* induction of tumor growth by hormones. The exact mechanism by which tyrosine kinases are involved in growth regulation is not known. It was suggested that these enzymes affect several metabolic pathways which might be related to each other. Among them are the phosphatidylinositol pathway [25], DNA and protein synthesis. Two recent reports link, albeit not directly, S6 phosphorylation and tyrosine kinase activity of the src and abl oncogene protein products [26,27]. The phosphorylation of this ribosomal protein, S6, was found to be involved in the regulation of protein synthesis. Another study suggests the enzymatic activity of topoisomerase as substrate for cytosolic tyrosine kinase in liver [28]. These and other reports challenge our findings and are presently under active study.

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