PROTEIN KINASE ACTIVITY IN MOUSE MAMMARY CARCINOMA

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SUMMARY

C3H mouse mammary carcinoma contains cyclic AMP-independent (C) and dependent (RC) protein kinases and a specific cyclic AMP-binding protein (R). The specific activities of C,RC and R are markedly lower in carcinoma than the normal mammary cells. Protein kinase preparation from neoplastic cells showed markedly higher ratio of C/RC and lower responsiveness to cyclic AMP for the activation of the enzyme than the normal cells.

Normal mammary cells differentiate into secretory cells which synthesize specific milk proteins in response to stimulation by insulin, hydrocortisone and prolactin (1). Mammary carcinoma cells of C3H mouse, however, fail to alter significantly their patterns of gene expression in this manner in response to these specific hormonal inducers(2,3).

Previous studies have characterised a mammary cytosol cyclic AMP-dependent protein kinase which plays a central role in proliferation and differentiation in mammary gland(4). The enzyme is induced rapidly in mammary gland in response to prolactin and it may subsequently mediate the action of the hormone by phosphorylating nuclear histones and specific phosphoproteins of plasma membranes and ribosomes(5-7). The present studies demonstrate the presence of a cyclic AMP-dependent protein kinase in C3H mouse mammary carcinoma and

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there is a marked alteration in the cyclic AMP responsiveness of these cells for the activation of protein kinase.

MATERIALS AND METHODS

Materials - [γ^{32}-P] ATP was obtained from International Chemical and Nuclear Corporation. Calf thymus histones were purchased from Worthington. DEAE-cellulose (Cellex D) was a product of BioRad. [8-^{3}H] Adenosine 3',5' -monophosphate (16.3 Ci per mmole) was from Schwartz BioResearch. Cyclic AMP was a product of Calbiochem and cyclic GMP, cyclic UMP and cyclic CMP were obtained from Boehringer-Mannheim.

Normal mammary gland was obtained from C3H/HeJ lactating mice(10-12 days post partum). C3H BA mouse mammary adenocarcinoma was maintained by serial transplantation in C3H/HeJ female hosts.

Isolation of mammary protein kinases - Mammary gland was homogenised in 0.15 M KCl containing 5 mM sodium glycerophosphate-HCl buffer (pH 6.5) and 0.2 mM dithiothreitol and the homogenate was centrifuged at 105,000 x g for 60 min to obtain cell supernate. The supernatant fraction was subjected to acid and ammonium sulfate fractionations and DEAE-cellulose chromatography by the method described earlier(4).

Assay of protein kinase - Cell supernate obtained by the procedure described above and isolated enzyme fractions were dialysed against 5 mM sodium glycerophosphate-HCl (pH 6.5) - 0.2 mM dithiothreitol prior to assay of protein kinase activity by the method described previously (6). A unit of enzyme activity was defined as the amount of enzyme which causes the transfer of 1 pmole of ^{32}P from [γ-^{32}P] ATP to the recovered protein during 30 min under the standard assay conditions.

Assay of cyclic AMP-binding protein - Cell supernatant fraction obtained by the procedure described above was dialysed against 5 mM sodium glycerophosphate-HCl (pH 6.5) - 0.2 mM dithiothreitol prior to assay of cyclic AMP-binding protein activity by the method described previously (5). A unit of cyclic AMP-binding protein activity has been defined as the amount of binding protein which binds 1 pmole of cyclic AMP under the standard assay conditions.

RESULTS AND DISCUSSION

Cyclic AMP-dependent protein kinase activity has been demonstrated in the cytosol fraction of mammary carcinoma cells, the specific activity of the enzyme being markedly lower (approximately 5-fold) in carcinoma than in the normal tissue (Table I). Carcinoma protein kinase preparation was

Table I

Protein kinase and cyclic AMP-binding protein activities in mouse mammary carcinoma and normal cells.

Activities of protein kinase and cyclic AMP-binding protein were measured by the procedures described in the text. The specific activities of these proteins were expressed on the basis of DNA contents in tissue homogenates as measured by the method of Burton(8).

Mammary gland	Protein kinase activity		Cyclic AMP-binding protein activity (R)	RC/R
	-Cyclic AMP	+ Cyclic AMP (RC)		
	units/mg DNA x 10^{-3}		units/mg DNA	
Normal	16.2	68.2	17.1	3.4
Carcinoma	4.5	13.3	6.0	1.5

activated to a lower extent (3-fold) than the enzyme preparation from the normal cells (4•2 - fold). Mammary carcinoma also showed much lower (approximately 3-fold) specific activity of the cyclic AMP-binding protein than the normal cells. The data further demonstrate that the ratios of cyclic AMP-dependent protein kinase and cyclic AMP-binding protein are 1.5 and 3.4 in neoplastic and normal cells respectively, indicating lower dependency of protein kinase activity on cyclic AMP during neoplasia.

Like the normal mammary gland, carcinoma cells as well showed the presence of two protein kinase peaks (I and II) as

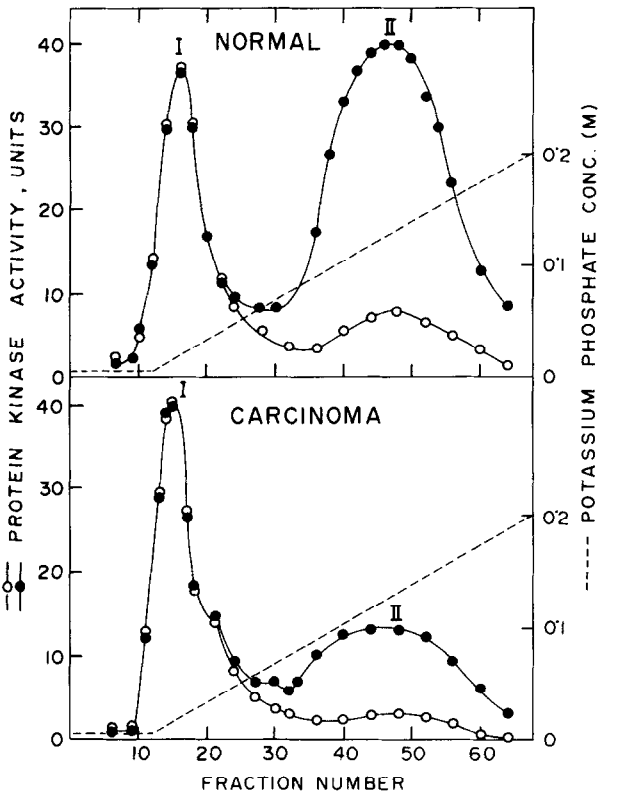

Fig. 1 Ammonium sulfate fractions of protein kinase obtained from 3 g. each of mouse mammary carcinoma and the normal tissue were applied to a DEAE - cellulose column (0.9 x 8.5 cm) previously equilibrated with 5 mm potassium phosphate buffer, pH 7.0 containing 2 mM EDTA. The column was washed with 15 ml of the equilibrating buffer prior to further elution with a linear gradient of potassium phosphate (5 to 500 mM) in a total volume of 200 ml of the buffer. Aliquots of 50 µl (carcinoma) and 25 µl (normal tissue) of each fraction (1.5 ml) were assayed for protein kinase activity with and without cyclic AMP (1 µM) under the standard assay conditions.

resolved by DEAE-cellulose chromatography(Fig.1). Activity of protein kinase I is independent of cyclic AMP whereas kinase II is markedly stimulated (approximately 5-fold) by 1 µM cyclic AMP. Chromatography profiles of the protein kinase are strikingly different in the normal and carcinoma cells. Kinase I and II represent approximately 25% and 75% respectively of total activity in normal tissue and approximately 50% each in malig-

nant tissue. Specific activities of kinase I and II are higher in normal cells than in carcinoma by approximately 2- and 6-fold respectively. Thus malignancy of mammary gland is associated with a marked increase in the ratio of cyclic AMP-independent and dependent protein kinase. Studies with various cyclic nucleotides : cyclic AMP, cyclic GMP, cyclic UMP and cyclic CMP demonstrated that the neoplastic mammary protein kinase II, like the normal mammary enzyme (4) is also activated specifically by cyclic AMP. No evidence has been obtained for the occurrence of cyclic GMP-dependent protein kinase in mammary carcinoma and normal cells.

Previous studies (6) demonstrated that mammary cyclic AMP-dependent protein kinase II (RC) is an inactive complex of a catalytic subunit (C) and a regulatory subunit (R) and cyclic AMP activates the enzyme by specifically binding with R, thereby releasing C which is the active form of the enzyme. Kinase I represents the catalytic subunit which can be converted to the cyclic AMP-dependent form (RC) after interaction with the specific cyclic AMP-binding protein(R). The observed lower responsiveness to cyclic AMP for the activation of carcinoma protein kinase may thus be attributed to higher ratio of C/RC in carcinoma than the normal cells.

Alteration in profile of the cyclic AMP-independent and dependent protein kinase species was also observed in hepatoma whereas no such alteration was detectable in sarcoma virus transformed cells(9-12). Hepatoma cells showed the presence of lower amount of RC, higher amount of C and lower responsiveness to cyclic AMP-activation of protein kinase as compared to normal liver cells(9,11). In normal mammary cells cyclic AMP-dependent protein kinase appears to play a pivotal role in the hormone-

dependent cell differentiation(5-7). It is possible that alteration of cyclic AMP responsiveness in the mammary carcinoma may cause a major change in the normal regulatory mechanisms in these cells.

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