Protein Phosphorylation in Normal and Neoplastic Development

CYCLIC AMP-DEPENDENT PROTEIN KINASE ACTIVITY IN URETHANE-INDUCED PULMONARY TUMOURS

By ALVIN M. MALKINSON, THOMAS J. GUNDERSON and CAROLYN E. McSWIGAN
*Dight Institute for Human Genetics and Department of Psychiatry, University of Minnesota,
Minneapolis, MN 55455, U.S.A.*

(Received 23 August 1977)

The cyclic AMP-dependent protein kinase specific activities of cytosol fractions from urethane-induced lung tumours and from normal mouse lung were determined. Both basal and cyclic AMP-stimulated activities in tumours were twofold higher than those of normal lung. Since neonatal and adult lung cytosols have identical kinase activities, the high activity in tumours appears to be a tumour-specific property.

The involvement of cyclic AMP in cancer [see Ryan & Heidrick (1974) for a review] mainly rests on two types of observations: an inverse correlation between intracellular cyclic AMP concentration and growth rate (Otten *et al.*, 1971), and a partial restoration of normal phenotype to transformed cells (Sheppard, 1971; Johnson *et al.*, 1971) and tumours (Cho-Chung, 1974) by exogenous addition of cyclic AMP. The only defined mechanism of action of cyclic AMP in eukaryotes is the activation of cyclic AMP-dependent protein kinase enzymes (EC 2.7.1.37) (Kuo & Greengard, 1969). An examination of these enzymes in malignant tissues therefore seemed warranted.

Previous studies have involved comparisons between normal and transformed cells or between hepatoma-cell lines and normal liver. No differences were reported in protein kinase activity between normal and transformed cells (Troy *et al.*, 1973; Gharrett *et al.*, 1976). Certain normal cells and their transformed counterparts contain different isoenzymes of protein kinase (Gharrett *et al.*, 1976), although others do not (Wehner *et al.*, 1977). The protein kinase isoenzymes of different hepatoma lines varied from each other (Criss & Morris, 1973) as well as from those found in normal liver (Mackenzie & Stellwagen, 1974; Granner, 1974). The physiological significance of the multiple forms of protein kinases has not yet been resolved. For example, mammalian hearts from different species contain different proportions of the two major isoenzyme classes (Corbin & Keely, 1977). It is clear that the intracellular concentration of protein kinase enzymes determines in part the sensitivity of a tissue to the effects of cyclic AMP (Beavo *et al.*, 1974).

A comparison between the protein kinases of tumours and of the normal tissues from which these tumours originated has not yet been made. In this

regard we describe such a comparison and use the specific activities of highly diluted protein kinase enzymes in the presence of an excess of substrate as an approach to determining protein kinase concentrations. The urethane-induced mouse pulmonary tumour was used as the test material since it is a well-characterized rapidly induced tumour [for a review see Shimkin & Stoner (1975)]. Because the same cell type that gives rise to these tumours also proliferates in response to various injuries to the lung (Bachofen & Weibel, 1974), this system seems well-suited for analysis of the role of cyclic AMP in the regulation of both normal growth and neoplastic development.

Strain-A/Umc mice, 6 weeks old, were injected intraperitoneally with 1 mg of 10% (w/v) urethane in 0.85% NaCl/g body wt. Control mice were not injected. At 12 or 16 weeks later mice were killed by cervical dislocation and the pearly-white lung tumours and the lung material free of visible tumours were collected and pooled separately. At least six experimental and two control mice were used per experiment. One or two tumour samples were fixed and histologically examined in each experiment to confirm the neoplastic nature of the dissected tumours. In one experiment, strain-A/St mice that had been injected with urethane 17 months previously were used.

The samples to be assayed were homogenized in 5 vol. of ice-cold 0.32M-sucrose, and cytosol fractions were prepared by using the final supernatant from the following consecutive centrifugations: 3000g, 5 min; 27000g, 15 min; 105000g, 45 min. Protein kinase activity was assayed in the presence or absence of 3.3 μ M-cyclic AMP by the method of Corbin & Reimann (1974) as modified by Gharrett *et al.* (1976). One unit of enzyme activity corresponded to the incorporation of 1 pmol of 32 P from [γ - 32 P]ATP into histone (type IIA mixture from

Table 1. *Cyclic AMP-dependent protein kinase activity in mouse pulmonary-tumour cytosol*

Results are means $\pm$ s.e.m. for seven independent experiments. The differences between tumour and controls for both basal and cyclic AMP-stimulated protein kinase activities were significant by Student's *t* test at $P < 0.001$.

	Protein kinase specific activity (units/mg of protein)		Activity ratio (-Cyclic AMP/+cyclic AMP)
	-Cyclic AMP	+Cyclic AMP	
Control	115 $\pm$ 25	509 $\pm$ 108	0.22
Uninvolved tissue	107 $\pm$ 12	574 $\pm$ 77	0.19
Tumour	235 $\pm$ 47	1079 $\pm$ 252	0.23

Sigma Chemical Co., St. Louis, MO, U.S.A.)/min at 30°C. The protein concentration was determined by the method of Lowry *et al.* (1951), with crystalline bovine serum albumin as a standard.

Only one of the 49 mice injected with urethane failed to show any pulmonary tumours. There was an average of 5.3 ± 4.5 (s.d.) tumours per mouse 12 weeks after urethane injections and 18.0 ± 6.6 tumours per mouse 16 weeks after injection. The A/St-strain mice that had been injected with urethane 17 months before being killed had 11 ± 5.4 tumours per mouse. Standard microscopic examination of tumours from each of these groups failed to show any major differences between them.

The protein kinase activities found in tumours, in the normal-appearing lung of urethane-injected animals and in the lungs of control animals are given in Table 1. The enzyme activities in tumours from animals injected 12 weeks, 16 weeks or 17 months before being killed were similar, and the results for all groups were therefore pooled. Cyclic AMP stimulated protein kinase activity approximately fivefold for all samples, as indicated by the activity ratios (activity measured in the absence of cyclic AMP/activity measured in the presence of cyclic AMP). The activities found in both control lungs and in uninvolved lung material were very similar. The activities of the tumour samples, however, were twice as high as the control lung samples. Since both basal and cyclic AMP-stimulated activities were higher in tumours than in the control samples, these increased kinase activities probably did not result from elevated intracellular cyclic AMP concentrations in the tumours. Rather, they suggest changes in the concentration of protein kinase enzymes or in the subcellular localization of the kinase enzymes (Corbin *et al.*, 1977).

Urethane-induced pulmonary tumours in mice are derived from type-II alveolar cells (Grady & Stewart, 1940). Crocker *et al.* (1970) showed that there is active proliferation of alveolar cells in the perinatal period. We have observed (A. M. Malkinson, L. Hoky, A. J. Gharrett & T. J. Gunderson, unpublished work) that the protein kinase specific activity of neonatal lung was identical with that of adult lung. It therefore appears that the elevated protein kinase

activity in the pulmonary tumours is a neoplastic characteristic rather than correlative of alveolar growth. This increased kinase activity could be one of the many alterations of metabolism found in pulmonary tumours (Greenstein, 1954) or it could suggest a regulatory role for this enzyme in determining or maintaining the neoplastic state. This is the first demonstration of differences in cyclic AMP-dependent protein kinase activity when a tumour was compared with its tissue of origin from within the same animal.

We thank Dr. J. C. Theiss (University of California, San Diego, CA, U.S.A.) for kindly supplying the A/St-strain mice, and Dr. J. J. Sauk, Jr. (University of Minnesota), for performing the microscopic examinations. This work was supported by a National Foundation-Basil O'Connor Grant and by United States Public Health Service grants CA 16228 and GM 22492.

References

- Bachofen, M. & Weibel, E. R. (1974) *Chest* **65**, 145-195
- Beavo, J. A., Bechtel, P. J. & Krebs, E. G. (1974) *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3580-3585
- Cho-Chung, Y. S. (1974) *Cancer Res.* **34**, 3492-3496
- Corbin, J. D. & Keely, S. L. (1977) *J. Biol. Chem.* **252**, 910-918
- Corbin, J. D. & Reimann, E. M. (1974) *Methods Enzymol.* **38**, 287-290
- Corbin, J. D., Sugden, P. H., Lincoln, T. M. & Keely, S. L. (1977) *J. Biol. Chem.* **252**, 3854-3861
- Criss, W. E. & Morris, H. P. (1973) *Biochem. Biophys. Res. Commun.* **54**, 380-386
- Crocker, T. T., Teeter, A. & Nielsen, B. (1970) *Cancer Res.* **30**, 357-361
- Gharrett, A. J., Malkinson, A. M. & Sheppard, J. R. (1976) *Nature (London)* **264**, 673-675
- Grady, H. G. & Stewart, H. L. (1940) *Am. J. Pathol.* **16**, 417-432
- Granner, D. K. (1974) *Arch. Biochem. Biophys.* **165**, 359-368
- Greenstein, J. P. (1954) *Biochemistry of Cancer*, 2nd edn., Academic Press, New York
- Johnson, G. S., Friedman, R. M. & Pastan, I. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 425-429
- Kuo, J. F. & Greengard, P. (1969) *Proc. Natl. Acad. Sci. U.S.A.* **64**, 1349-1355

- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mackenzie, C. W., III & Stellwagen, R. H. (1974) *J. Biol. Chem.* **249**, 5755–5762
- Otten, J., Johnson, G. S. & Pastan, I. (1971) *Biochem. Biophys. Res. Commun.* **44**, 1192–1198
- Ryan, W. L. & Heidrick, M. L. (1974) *Adv. Cyclic Nucleotide Res.* **4**, 81–116
- Sheppard, J. R. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 1316–1320
- Shimkin, M. B. & Stoner, G. D. (1975) *Adv. Cancer Res.* **21**, 1–58
- Troy, G., Vijay, I. K. & Kawakami, T. G. (1973) *Biochem. Biophys. Res. Commun.* **52**, 150–157
- Wehner, J. M., Malkinson, A. M. & Sheppard, J. R. (1977) *Adv. Cyclic Nucleotide Res.* **9**, in the press