Decreased Expression of the Type I Isozyme of cAMP-dependent Protein Kinase in Tumor Cell Lines of Lung Epithelial Origin*

(Received for publication, December 7, 1989)

Carol A. Lange-Carter,† Tellef Fossli,† Tore Jahnssen,§ and Alvin M. Malkinson††

From the †Molecular and Environmental Toxicology Program, School of Pharmacy, University of Colorado, Boulder, Colorado 80309 and the §Institute of Pathology, Rikshospitalet, 0027 Oslo 1, Norway

A spontaneous transformant derived from a mouse lung epithelial cell line exhibited decreased cAMP-dependent protein kinase (PKA) activity. DEAE column chromatography demonstrated that this was caused by specific loss of the type I PKA isozyme (PKA I). Western immunoblot analysis indicated that indeed several mouse lung tumor-derived cell lines and spontaneous transformants of immortalized, nontumorigenic lung cell lines contained less PKA I regulatory subunit (R1) protein than normal cell lines. PKA II regulatory subunit protein differed only slightly among cell lines and showed no conspicuous trend between normal and neoplastic cells. The decrease in R1 was apparently concomitant with decreased catalytic (C) subunit levels in neoplastic cells since no free catalytic subunit activity was detected by DEAE chromatography. Northern blot analysis using Rα and Cα cDNA probes showed that the levels of Rα and Cα mRNAs paralleled their intracellular protein concentrations; neoplastic cell lines contained significantly less Rα and Cα mRNAs than the normal cell line. The decreased expression of both R1 and C subunits therefore results in a net decrease of PKA I in neoplastic lung cells, an isozyomic difference which may account for the differential effects of cAMP analogs on cell growth and differentiation in normal and neoplastic cells.

cAMP inhibits the growth of several mammalian cell types (Russell, 1978; Cho-Chung, 1989), but stimulates proliferation of other cells (Dumont et al., 1989). The opposite effects of cAMP may reflect the cell-specific concentration, types, and/or localization of intracellular cAMP receptors. Physiological responses to cAMP are mediated by two classes of cAMP-binding proteins, the regulatory (R) and catalytic (C) subunits of type I and II cAMP-dependent protein kinases (PKA I and PKA II, respectively) (Corbin et al., 1975). Two distinct gene products, termed α and β, exist for both R1 and R2, whereas three gene products have been identified for the catalytic (C) subunit (α, β, γ; Øyen et al., 1989). Expression of the different R and C subunits is tissue-specific (Clegg et al., 1988) and hormonally regulated (Hatoosh et al., 1987; Øyen et al., 1988a, 1988b). For example, Rα is expressed constitutively in most tissues, whereas Rβ is detected only in brain, spinal cord, and testis (Clegg et al., 1988).

An impaired ability to produce cAMP in response to extracellular signals and a reduced responsiveness of PKA to cAMP are associated with neoplastic transformation of mouse lung epithelium, an animal model of human bronchiolo-alveolar carcinoma (Malkinson, 1989). Both spontaneous in vitro transformants and cell lines derived from urethan-induced lung tumors exhibited decreased isoproterenol-stimulated cAMP accumulation, probably as a result of a functionally altered Gs complex (Droux et al., 1989). Chemically induced mouse lung tumors contain functionally altered Gs proteins as reflected by their decreased ability to incorporate the photoaffinity analog 8-azido-GTP (Droms et al., 1987). Rγ from urethan-induced lung tumors lacks high affinity cAMP-binding sites, and PKA II from these tumors is less responsive to dissociation by cAMP (Malkinson and Butley, 1981; Butley et al., 1984, 1985). During advanced stages of tumor progression, less of the phosphorylated form of Rγ is present, and R subunits are partially degraded to 37-kDa proteolytic fragments due to increased activity of a Ca++-activated protease which catalyzes R subunit digestion (Butley et al., 1985).

Smith et al. (1984) have cloned immortalized nontumorigenic cell lines from normal mouse lung epithelium and obtained tumorigenic variants (spontaneous transformants) of these cell lines by selecting for cells able to grow in the presence of dexamethasone (Smith and Lykke, 1985). The great advantage of these lines is that one has a control cell (the nontumorigenic line) with which to compare characteristics of the neoplastic cell lines. We have compared PKA isozyme expression at the mRNA and protein levels between these normal cells and their neoplastic derivatives.

THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 1990 by The American Society for Biochemistry and Molecular Biology, Inc.
Proven in U.S.A.
PKA Activity—Cells were scraped into cold saline, pelleted by a 5-min centrifugation at 135 × g, and homogenized in 5 volumes of buffer (0.32 M sucrose, 10 mM potassium phosphate (pH 7.4), 1 mM EDTA, 50 μg/ml leupeptin, 2 mM phenylmethylsulfonyl fluoride). Postmitochondrial supernatant (cytosolic) fractions were prepared by a 30-min centrifugation of 20,000 × g, and the recovered pellet was resuspended in the original volume of buffer. PKA activity was measured by incorporation of the γ-phosphate from [γ-32P]ATP into the oligopeptide substrate Kemptide (Roskoski, 1983).

Chromatography—Cytosolic fractions containing 5–8 mg of protein were preincubated in 0.15 mM ATP and 3.5 mM MgCl2 at 30 °C for 30 min to facilitate association of the PKA I holoenzyme and passed over a Sephadex G-25 column (30 × 0.9 cm). Void volume fractions were then pooled and subjected to DEAE-cellulose column chromatography as described (Malkinson et al., 1978a). Column fractions were stored at 4 °C, and PKA activity was assayed within 8–12 h post-elution.

8-N3[^2P]cAMP Photolabeling—PKA R subunits in DEAE-cellulose column fractions were photolabeled as previously described (Butley et al., 1984), except that the concentration of 8-N3[^2P]cAMP used was 750 nM.

RESULTS

PKA Activity in Normal and Neoplastic Lung Cell Lines—Multiple anomalies in the cAMP signal transduction pathway were observed at various stages during lung tumor development (Malkinson and Butley, 1981; Butley et al., 1984, 1985; Droms et al., 1987, 1989) prompted us to examine PKA enzymes in normal and neoplastic cell lines derived from mouse lung epithelium. Both cytosolic and particulate PKA activities in normal C10 cells were higher (P < 0.001 by Student’s t test) than the corresponding fractions from neoplastic E9 cells (Table I). These PKA-specific activities are of the same order of magnitude as those found in cytosolic and particulate extracts prepared from lung tissue.

PKA Isozyme Composition in Normal and Neoplastic Lung

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cytosol</th>
<th>Particulate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+cAMP</td>
<td>-cAMP</td>
</tr>
<tr>
<td>C10</td>
<td>4110 ± 140</td>
<td>300 ± 50</td>
</tr>
<tr>
<td>E9</td>
<td>2100 ± 80</td>
<td>100 ± 9</td>
</tr>
</tbody>
</table>

FIG. 1. DEAE-cellulose chromatography of normal C10 and neoplastic E9 cells. Separate columns were loaded with 7.8 mg of protein prepared from the cytosolic fraction of each cell line as described under “Materials and Methods.” PKA activity in the presence of 3.3 μM cAMP was measured using 25-μl aliquots of column fractions eluted from C10 (O) and E9 (○) cells. NaCl (○)—○ concentrations (right-hand scale) were determined by conductivity measurements of each fraction. PKA activity due to free C subunits elutes in the void volume when present, but was not detected in these studies. Kinase activity in both isozyme peaks were nearly completely inhibited upon addition of 0.1 μg/μl PKI (Ashby and Walsh, 1972), demonstrating that this activity was PKA-specific. Inset, 8-N3[^2P]cAMP photolabeling of R subunits in DEAE column fractions from normal C10 and neoplastic E9 cells. Twenty-five-μl aliquots from even-numbered DEAE-column fractions (fractions 12–58) eluted from C10 (top) and E9 (bottom) cells were photolabeled with 8-N3[^2P]cAMP.

Cell Lines—Because total cellular PKA-specific activity reflects the contribution of both isozymes, PKA isozyme composition was examined by DEAE-cellulose chromatography. PKA activity from C10 and E9 cells eluted in two distinct peaks at 0.02–0.10 and 0.17–0.23 M NaCl, respectively (Fig. 1), consistent with elution positions of adult mouse lung PKA isozymes (Malkinson et al., 1978b; Butley and Malkinson, 1984). The cAMP-dependent protein kinase activities in both isozyme peaks were inhibitable by the specific PKA inhibitor PKI. Because PKA I from neonatal mouse lung epithelial cells eluted at the salt concentrations characteristic of both PKA I and PKA II (Butley and Malkinson, 1984), isozyme positions were validated by photolabeling R and R in the eluted fractions with 8-N3[^2P]cAMP (Fig. 1, inset). The amount of photolabeled R in low salt peak (fractions 14–52) column fractions was much greater in C10 cells than in corresponding fractions from E9 cells. The high salt peak (fractions 38–52) contained R and the 37-kDa proteolytic product of PKA. Trace amounts of R in these fractions, consistent with the elution characteristics of normal adult mouse lung PKA (Butley et al., 1984). The relative amounts of R and R in the 37-kDa fragment in these high salt fractions were approximately equal in both cell lines. Increased degradation of R subunits to the 37-kDa fragment has been com-
monly observed in high salt DEAE-cellulose column fractions from neonatal mouse lung tissues and tumors (Butley and Malkinson, 1984; Butley et al., 1985). The cAMP dependence of the protein kinase activity peaks using the Kemp-tide oligopeptide as substrate attests to the holoenzyme state of the eluted PKA isozymes.

The PKA I activity peak from neoplastic E9 cell extracts is much smaller than that from normal C10 cells (Fig. 1, fractions 12–30). The absence of Kempktide kinase activity elsewhere in the eluate or void volume implies that C subunit as well as R1 concentrations in E9 cells were low. Coordinate control of R and C subunit expression has been observed in other systems (Weber and Hilz, 1986; Breton et al., 1989). Integration of peak areas in independent experiments typically indicated the presence of 4–5-fold more PKA I in C10 cells than in E9 cells, whereas the PKA II holoenzyme peak (fractions 38–52) areas were approximately equal in both cell lines.

$R_1$ and $R_1$ Protein Concentrations in Normal and Neoplastic Lung Cell Lines—To address the mechanism of reduced PKA I activity in neoplastic cells and to ascertain whether this defect was a general phenomenon, $R_1$ concentration was examined in several lung cell lines by Western blot analysis of cell extracts. Rabbit anti-rat $R_1$ antibody detected a 49-kDa protein (Fig. 2A) which co-migrated with purified rat brain $R_1$ (lane 7). Calibration curves constructed via microdensitometry demonstrated a linear relationship between the amount of protein loaded onto each gel lane and the amount of $R_1$ protein detected by Western blot. Normal C10 and E10 cells contained the most $R_1$ protein (lanes 3 and 6, respectively). Neoplastic cell lines E9, PCC4, A5, and 82-132 (lanes 1, 2, 4, and 5, respectively) exhibited 3–4-fold less $R_1$ protein compared to normal cells.

To determine whether $R_1$ protein expression was altered in neoplastic cell lines compared to normal cell lines, $R_1$ protein levels were also examined by Western immunoblot analysis (Fig. 2B). Rabbit anti-bovine $R_1$ antibody detected a protein doublet consisting of two forms of $R_1$ (54 and 56 kDa); the slower form co-migrated with purified bovine heart $R_1$ (lane 7). Both of these immunoreactive forms bind 8-Ne$^{33}$P-cAMP. The 56-kDa band most likely represents the autophosphorylated form of $R_1$ ($R_1$-P) (Butley et al., 1984, 1985). $R_1$-P is the predominant form found in many tissues (Rangel-Aldao et al., 1979; Scott and Mumby, 1989), whereas $R_1$-P is more abundant than $R_1$-P in most cultured lung cell lines. Although some clonal variation in $R_1$ expression was apparent between cell lines, no consistent differences in total $R_1$ in normal (lanes 1 and 3) versus neoplastic (lanes 2 and 4–6) cell lines were indicated by $R_1$ protein immunoblots.

Expression of $R_1$, and $R_2$ mRNAs in Normal and Neoplastic Lung Cell Lines—To determine the cause of reduced $R_1$ protein in neoplastic cells, $R_1$ mRNA concentrations were examined using a cDNA probe for $R_1$. Equal amounts of total RNA from each cell line were loaded onto the gel. The $R_1$ cDNA probe detected an mRNA band of 3.2 kilobases (Fig. 3). Normal C10 cells contained the most $R_1$ message (lane 3) of several cell lines tested; faint bands of $R_2$ mRNA from the neoplastic cell lines are discernible on the original autoradiogram. Neoplastic PCC4 and 82-132 cell lines derived from lung tumors (lanes 2 and 5, respectively) and the E9 and A5 spontaneous transformants (lanes 1 and 4, respectively) of normal E10 and C10 cells, respectively, all contained much less $R_2$ mRNA than C10 cells. Because E9 cells had less cAMP-dependent protein kinase activity than C10 cells, $C_1$ subunit mRNA concentrations in these cell lines were also examined. A single mRNA band of 2.4 kilobases in size was detected by the $C_1$ cDNA probe (Fig. 4). The apparent $C_1$ mRNA concentration was greatly diminished in neoplastic E9 cells (lane 2) compared to normal C10 cells (lane 1). Expression of both $R_1$ and $C_1$ subunits is thus depressed in E9 cells.

Northern analysis using $R_1$ and $R_2$ cDNA probes disclosed no apparent differences between normal and neoplastic cell lines (data not shown).

DISCUSSION

This is the first demonstration of an absolute decrease in the cellular concentration of the PKA I subunits and their mRNAs as a result of neoplasia. The fact that this deficiency was found in both spontaneous transformants and cell lines derived from carcinogen-induced and spontaneous tumors.

2 C. A. Lange-Carter and A. M. Malkinson, unpublished results.
attests to the generality of this phenomenon in mouse lung cancer and implies that this PKA alteration is reflective of the neoplastic state rather than some eccentricity of one particular cell line. These tumorigenic mouse lung cell lines with greatly reduced PKA I content represent an intriguing model system for elucidating the physiological consequences of altered PKA isozyme expression. The specific activity of protein kinase C is also reduced in one of these neoplastic lung epithelial cell lines (Nicks et al., 1989). Thus, the activities of the two protein kinases which act as distal effectors for the major second messengers, cAMP and diacylglycerol, are both decreased, making these cells less responsive to agents whose actions are mediated by these second messengers, including growth inhibitory signals.

The mechanism of decreased PKA I expression in neoplastic lung cells may involve differential mRNA stability or altered transcription rates of Rr and CI subunits. Further insight may be gained by experimental manipulation of PKA subunit levels. Treatment of neuroblastoma-glioma hybrid cells with dibutyryl cAMP, prostaglandin E₂, or 3-isobutyl-1-methylxanthine raised Rr levels, but did not affect Rᵣ₀ or PKA activity levels (Walter et al., 1979). Rᵣ₀ but not Rᵣᵣ, increased when N-18 mouse neuroblastoma cells were grown in medium containing 1% rather than 10% fetal calf serum (Liu et al., 1980). Those studies assessed R subunit levels using 8-N₂-[³²P] cAMP incorporation rather than by immunoblotting techniques as used herein. Addition of dibutyryl cAMP to primary Sertoli cell cultures for 14 and 24 h induced Rᵣᵣ mRNA levels 50-60-fold, whereas continued stimulation for 36 h and 48 h was associated with a time-dependent decrease (Øyen et al., 1988a, 1988b), an example of biphasic control of PKA subunit expression by cAMP. Low serum concentrations increased Rᵣᵣ levels, whereas addition of 100 μM forskolin to cells growing in low serum decreased Rᵣᵣ levels in both C10 and E9 cells; the Rᵣᵣ level in E9 cells always remained much lower than in C10 cells.

Decreased levels of one PKA subunit may cause a decreased intracellular concentration of the other subunit. Kin⁻ S49 lymphoma mutants lacking C subunits contain low levels of Rᵣᵣ probably as a result of enhanced degradation of free Rᵣᵣ subunits (Steinberg and Agard, 1981). Elevation of C subunit levels in NIH-3T3 cells by transfection with either Ca or Cp cDNA expression vectors increased the level of RI protein, but not that of Rᵣᵣ protein, suggesting that RI is stabilized by the C subunit in the holoenzyme complex (Uhler and McCormick, 1987). Coordinate control of R and C subunit concentrations may ultimately reside at the mRNA level. PKA subunit levels in neoplastic mouse lung appear to be determined by their corresponding mRNA levels.

Selective modulation of PKA isozymes may underlie cAMP-mediated regulation of cell growth and differentiation; however, no specific role in these processes has yet been assigned to each PKA isozyme (Beebe and Corbin, 1986). This is in part due to the complexity of cAMP mediation of cellular events. PKA I increased during isoproterenol-induced cardiac hypertrophy (Byus et al., 1976) and became activated in early lymphocyte mitogenesis (Byus et al., 1977), implicating Rᵣᵣ as a positive effector of growth. Virally transformed 3T3 fibroblasts contain PKA I, whereas untransformed 3T3 cells do not (Gharrett et al., 1976); normal fibroblasts from other species contain both isozymes, however (Ray et al., 1979; Wehner et al., 1981). Divergent PKA isozyme composition for a single organ across many species was most dramatically illustrated for heart (Corbin and Keely, 1977). 8-Cl-cAMP inhibited human colon cancer growth, and this was associated with changes in the levels and cellular localization of both Rᵣᵣ and Rᵣᵣ (Ally et al., 1988). PKA I activity and protein levels increased in aging IMR-90 cells (over 48 population doublings) compared to younger cells (20-27 doublings), with no significant differences in PKA between logarithmic and stationary phase cells (Liu et al., 1986). We also found no differences in Rᵣᵣ mRNA and protein expression between growing versus confluent cell cultures.

Acknowledgments—We gratefully acknowledge the technical assistance of Kendal M. Nicks and helpful comments on the manuscript by Dr. Kurt A. Droms.

REFERENCES


Malkinson, A. M. (1986) Cancer Res. 46, 5191-5198


Decreased Expression of PKA I in Lung Neoplasia


Decreased expression of the type I isozyme of cAMP-dependent protein kinase in tumor cell lines of lung epithelial origin.

C A Lange-Carter, T Fossli, T Jahnsen and A M Malkinson


Access the most updated version of this article at http://www.jbc.org/content/265/14/7814

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/265/14/7814.full.html#ref-list-1