Terminal neuroendocrine differentiation of human prostate carcinoma cells in response to increased intracellular cyclic AMP

(hormone-refractory/pp60$^{c\text{-}src}$/multipotential)

Y.J. BANG*[†], T, F. PIRNIA*, W.G. FANG*[‡] , W. K. KANG*[†], O. SARTOR*, L. WHITESELL*, M. J. HA*,
M. TSOKOS[§], M. D. SHEAHAN*, P. NGUYEN*, W. T. NIKLINSKI*, C. E. MYERS*, AND J. B. TREPEL*[¶]

*Clinical Pharmacology Branch, Clinical Oncology Program, and [§]laboratory of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892
[†]Present address: Department of Internal Medicine, Seoul National University Hospital, Seoul, 110744, South Korea.
[‡]Present address: Department of Pathology, Beijing Medical University, Beijing 100083, China.
[¶]To whom reprint requests should be addressed at: Clinical Pharmacology Branch, National Cancer Institute, Building 10, Room 12N226, National Institutes of Health, Bethesda, MD 20892.

Communicated by Leonard S. Herzenberg, December 6, 1993 (received for review August 20, 1993)

Abbreviations: db, dibutyryl; TGF, transforming growth factor; IBMX, isobutylmethylxanthine; NSE, neuronspecific enolase.

ABSTRACT: Recent clinicopathologic studies have shown that many prostatic andenocarcinomas express focal neuroendocrine differentiation and that neuroendocrine differentiation is most apparent in advanced anaplastic tumors. While studying growthregulatory signal transduction events in human prostate carcinoma cell lines, we found that in two of four cell lines, the androgen-sensitive line LNCaP and the highly metastatic androgenindependent line PC3M, elevation of cAMP through addition of cAMP analogues or phosphodiesterase inhibitors induced a markedly neuronal morphology. Also in LNCaP cells ultrastructural analysis showed that cAMP induced the appearance of neurosecretory cell-like dense-core granules. Phenotypic analysis of untreated LNCaP and PC3M calls shoved that both cell lines express markers of the neural crest including S100, cromogranin A, pp60$^{c\text{-}src}$, and neuron-specific enolase as well as the epithelial marker KSl/4 and stagespecific embryonic antigen 4. In PC3M cells, cAMP markedly elevated neuron-specific enolase protein and caused an increase in the specific activity of the neuroendocrine marker pp60 $^{c\text{-}src}$, and in both cell lines expression of KS1/4 and stage-specific embryonic antigen 4 was down-regulated. In addition to effects on lineage markers, cAMP treatment induced G_l synchronization, growth arrest, and loss of clonogenicity, indicating terminal differentiation. Our data provide direct evidence of plasticity in the lineage commitment of adenocarcinoma of the prostate. We have shown that cell-permeant cAMP analogies can induce terminal differentiation, suggesting that hydrolysisresistant cyclic nucleotides may present an additional approach to the treatment of advanced prostate cancer.

In an effort to develop new anticancer drugs directed at unique aspects of prostate cancer biology, we have been studying the signal transduction pathways regulating the growth of human prostate adenoeareinoma cells *in vitro.* As reported previously, we found that addition of dibutyryl (db) cAMP to the androgenindependent prostate carcinoma cell line PC3 causes induction of type ß2 transforming growth factor (TGF ß2) mRNA, production of bioactive TGF ß2, and growth arrest (1).

We have subsequently studied the effect of eAMP derivatives and phosphodiesterase inhibitors on the other two commonly available prostate carcinoma cell lines, DU 145 and LNCaP, as well as the highly metastatic variant of PC3, PC3M, and found that ail lines were growth inhibited by elevation of intracellular eAMP (data not shown). Data presented here demonstrate that in two of these lines, LNCaP and PC3M, elevation of intracellular eAMP induces permanent conversion from an epithelial to a neuronal morphology and that these cells express markers of the neuroendocrine phenotype. These data suggest that these cell lines, which are derived from metastatic adenocarcinoma of the prostate, contain or consist of multipotent cells capable of both neuroendocrine and epithelial differentiation.

MATERIALS AND METHODS

Cell Culture. The human prostate carcinoma cell lines PC3, DU 145, and LNCaP were obtained from the American Type Culture Collection. PC3M, a highly metastatic variant of PC3 cells (2), was a kind gift of James Kozlowski (Department of Urology, Northwestern University). LNCaP and PC3M cells were maintained in RPMI 1640 medium with 10% fetal bovine serum. Treatment with dbeAMP or isobutylmethylxanthine (IBMX) was started 24 hr after subculturing cells and continued every other day. SKNAS neuroblastoma cells (3) were kindly provided by Lee Helman (Pediatric Branch, National Cancer Institute).

Growth Studies. Cells were seeded at an initial density of 5000 cells per cm^2, incubated for 24 hr. and treated as indicated. Viable cell number was determined by hemacytometer counts of trypan blue-excluding cells. For clonogenic assays, cells were treated for 6 days, harvested, washed twice, replated in fresh medium at the same viable cell number per plate, and grown in the absence of additional drug. After 7 days, the cells were stained with methylene blue and colonies were counted using an Artek 880 automated colony counter.

Electron Microscopy. For transmission electron microscopy, the cells were fixed *in situ* with glutaraldehyde, embedded in agar, postfixed in osmium tetroxide, stained *en bloc* with uranyl acetate, dehydrated, infiltrated with plastic, embedded and polymerized, sectioned 70 nm thick, poststained with lead citrate, and examined in a Zeiss EM 10/C electron microscope. For scanning electron microscopy, the cells were fixed *in situ* with glutaraldehyde, dehydrated, criticalpoint dried, coated with gold palladium, and examined in an Amray 1820 scanning electron microscope.

Immuocytochemistry. Control and cAMPtreated cells were fixed with acetone at 4°C for 10 min. Rabbit antibodies recognizing human neuronspecific enolase (NSE) and S-100 protein were obtained from Dako. Antibody reactivity was detected by using biotinylated $F(ab')_2$ antirabbit immunoglobulin and streptavidinconjugated horseradish peroxidase, followed by incubation with hydrogen peroxide and the chromogen aminoethylcarbazole (Tago).

Four Cytometry. The epithelial antigen KS1/4 and stage-specific embryonic antigen expression were analyzed by flow cytometry (4). For cell cycle analysis, cells were harvested, fixed in 50% ethanol, and incubated with the DNA intercalating dye chromomycin A (Sigma).

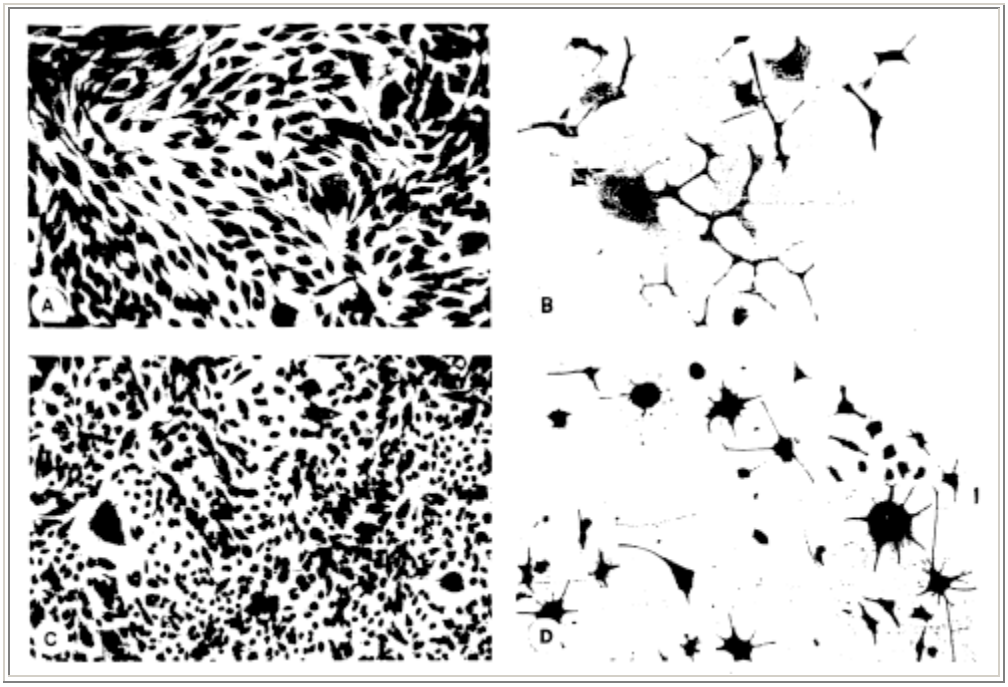
Western Blot Alkali. Cell lysates were subjected to electrophoresis in a SDS/7.5% polyacrylamide gel; proteins were transferred onto nitrocellulose paper, blocked, and incubated with monoclonal antihuman NSE antibody or monoclonal antichromogranin A antibody (Boehringer Mannheim). NSE immunoblots were washed and incubated with ^{125}Iconjugated goat antimouse immunoglobulin, washed, dried, and subjected to autoradiography. Chromogranin A immunoblots were incubated with horseradish peroxidaseconjugated rabbit antimouse immunoglobulin and developed with a chemiluminescent substrate (ECL, Amersham).

Immune Complex Kinase Assay of pp60$^{c\text{-}src}$ Activity. pp60$^{c\text{-}src}$ was immunoprecipitated with mouse monoclonal antibody 327 (5) and protein ASepharose beads coated with rabbit antimouse immunoglobulin and incubated with [λ^{32}P]ATP (DuPont/NEN) and the exogenous substrate rabbit muscle enolase (Sigma). Proteins were analyzed by SDS/PAGE followed by autoradiography. Monoclonal antibody 327 was a kind gift of Joan Brugge (ARIAD Pharmaceuticals, Cambridge, MA).

RESULTS

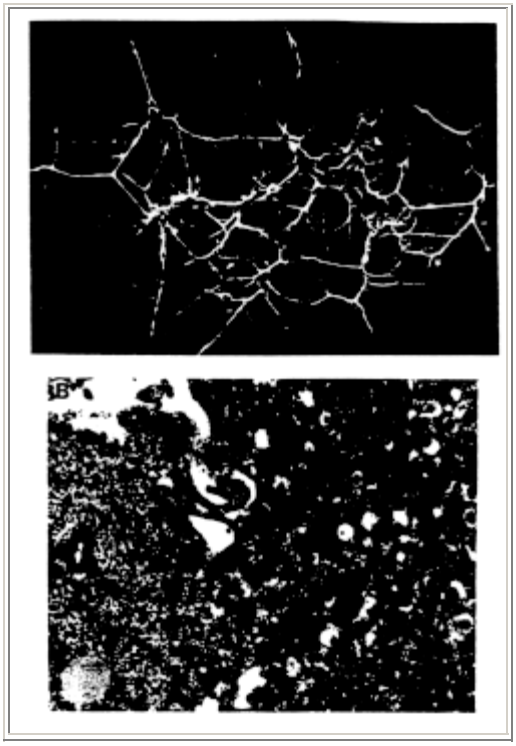
Effects of Elevating cAMP on Prostate Carcinoma Morphology. After addition of dbcAMP or IBMX to each of the four prostate carcinoma cell lines studied two of the cell lines, DU 145 and PC3, showed little change in morphology (data not shown). In contrast, LNCaP and PC3M cells developed features of neuronal morphology after addition of either dbcAMP or IBMX. Fig. 1 *A* and *B* shows the effect of addition of dbcAMP plus IBMX on PC3M cells and Fig. 1 *C* and *D* shows the effect of this treatment on LNCaP cells. Untreated cells had an epithelial morphology and tended to grow in clusters with a somewhat acinar appearance. In contrast, dbcAMP or IBMXtreated cells had a neuronal appearance, characterized by bipolar or multipolar cells with small cell bodies, long processes, and beaded varicosities. Equivalent morphologic effects were seen with a variety of phosphodiesterase inhibitors, including pentoxiphylline and theophylline, and with other cellpermeant cAMP analogs, including phosphorothioatemodified (Sp isomer) cAMP (data not shown). Thus, the effects of dbcAMP did not require the presence of the butyrate moiety. Treatment with dbcAMP alone was a sufficient stimulus to induce all or most of the effects we report (data not shown). The ability of dbcAMP to elevate intracellular cAMP levels in LNCaP and PC3M cells was verified by using a ^{125}Ilabeled cAMP radioimmunoassay (DuPont) (data not shown).

FIG. 1. Wright-stained PC-3-M and LNCaP cells. (*A*) Untreated PC-3-M cells. (*B*) PC-3-M cells treated for three days with 1 mM db-cAMP/500 μM IBMX. (*C*) Untreated LNCP cells. (D) LNCP cells treated for 5 days with 1 mM db-cAMP/500 μM IMBX.



As shown in the scanning electron micrograph of LNCaP cells treated with dbcAMP plus IBMX (Fig. 2*A*), rather than growing in glandularappearing clusters, the cells developed a neuronal morphology in which each cell body is isolated, and multipolar processes form contacts with processes from neighboring cells.

FIG. 2. (*A*) Scanning electron micrograph of LNCaP cells treated with 1 mM db-cAMP/500 mM IBMX. (*B*) Transmission electron micrograph of LNCaP cells treated with 1 mM dbcAMP/500 µM IBMX for 6 days. (X 22,500.)

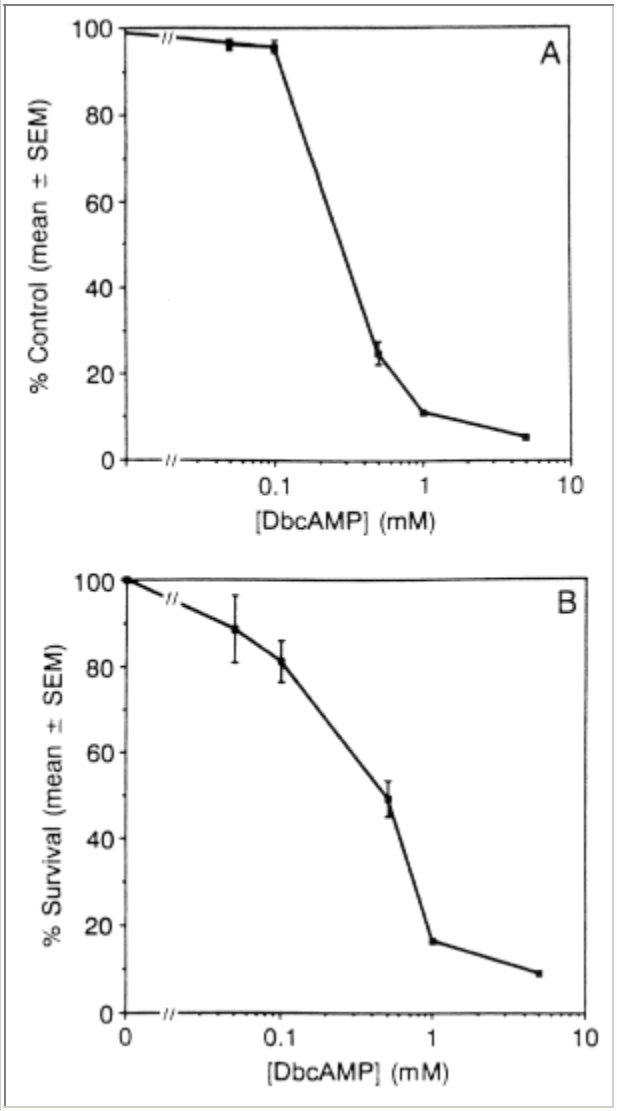


The cell lines were further characterized by transmission electron microscopy. Untreated cells were flat and elongated without evidence of neural features except that myelinlike structures were weakly evident in PC3M and LNCaP (data not shown). After treatment with dbcAMP and IBMX, PC3M and LNCaP were more rounded and, as shown in Fig. 2*B*, LNCaP developed numerous double membrane-bound densecore granules, characteristic of well differentiated neurosecretory cells. Elevation of intracellular cAMP was not a sufficient signal for induction of densecore vesicles in PC3M cells. Desmosomes and intracytoplasmic lumina, which are typical of epithelial cells, were evident in both cell lines before and after treatment (data not shown), providing ultrastructural support for the biphenotypic nature of these cells.

cAMP Induces Growth Arrest and Loss of Clonogenicity. The concentration dependence of the effect of dbcAMP on the growth of PC3M cells is shown in Fig. 3*A*. The IC_{50} for dbcAMP was 500 μM. Comparable curves were seen in the LNCaP cell line (data not shown). Several other cAMP analogues were tested on PC3M cells and the most effective in inducing growth arrest was phosphorothioate-modified cAMP, which had an IC_{50} of 33 μM. Fig. 3*B* shows the results of clonogenic assays on db-cAMPtreated PC3M cells. There was a marked decrease in clonogenicity after dbcAMP treatment. Because this experiment is performed by treating the cells with dbcAMP, washing, and replating in the absence of dbcAMP, the growth arrest and loss of clonogenicity is a stable phenotypic alteration. Flow cytometric cell cycle analysis showed that dbcAMPtreated cells were arrested in G_o/G_1 (data not shown).

FIG. 3. *(A)* Concentration dependence of db-cAMPinduced growth inhibition in PC3M cells. Data are expressed as percentage of the number of viable cells in untreated control wells. Data are representative of three different experiments and values are means ± SEM (n = 3). (*B*) cAMPinduced loss of

clonogenicity in PC3M cells. Data represent means ± SEM of three different experiments.

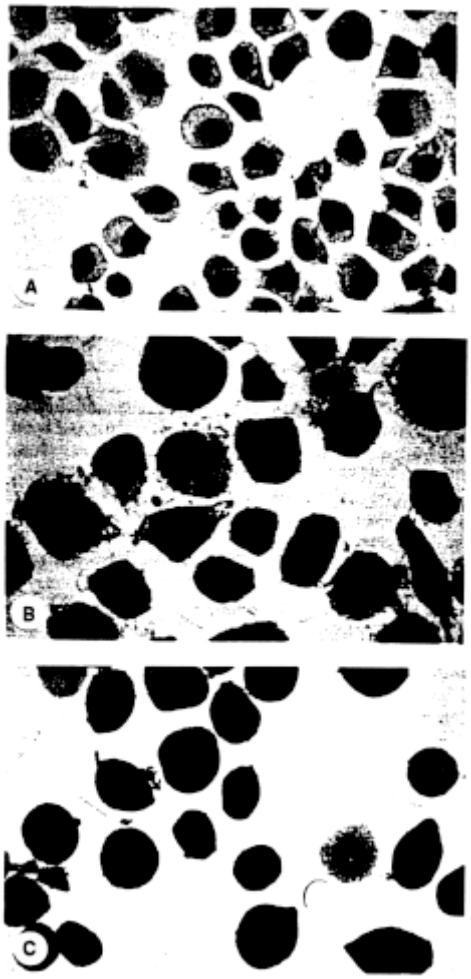


Expression of Neuroendocrine Markers. Immunocytochemical and Western blot analyses were used to evaluate the expression of neuroendocrine markers in untreated cells and after *in vitro* differentiation with dbcAMP and IBMX. The markers chosen for initial immunologic studies were NSE, S100, chromogranin A, and $pp60^{c\text{-}src}$. Untreated PC3M cells showed intense NSE expression by cytochemical analysis (Fig. 4*B*). There was low NSE expression in LNCaP cells (data not shown). An advantage of NSE as a marker is that monoclonal antiNSE antibody can be used to detect NSE on Western blots, allowing identification of the molecular mass of the protein recognized cytochemically. NSE is a homodimeric protein composed of monomers with a relative mobility corresponding to a molecular

mass of 46 kDa (6). In Western blot analysis of PC3M cells, NSE ran as a single band of 47 kDa. After 6 days of treatment with dbcAMP and IBMX, there was a marked increase in the level of NSE protein (Fig. 5*A*).

FIG. 4. Immunocytochemical analysis of PC3M cytospin

preparations. (*A*) Isotype control antibody. *(B)* Antibody to NSE. (*C*) Antibody to S100.

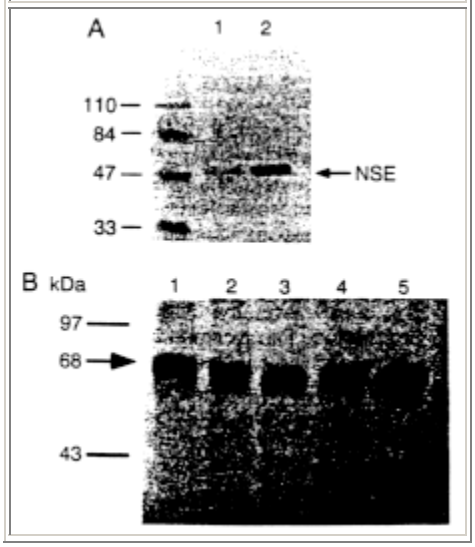


Cytochemical analysis of untreated PC3M cells showed very strong expression of the neuroendocrine and neural crest marker S100 (7) (Fig. 4*C*). LNCaP cells were S100 negative, and the levels of S100 did not change discernibly after dbcAMP treatment of PC3M or LNCaP (data not shown). The presence of S100 in PC3M cells and the observation of myelinlike structures, characteristic of glial cells, in PC3M and LNCaP cells suggest that there may be evidence of multipotential differentiation (glial as well as neuronal and epithelial) in prostatic carcinoma cells.

Both LNCaP and PC3M cells when untreated expressed the neuroendocrine marker chromogranin A, which was originally isolated from chromaffin granules of adrenal medulla, and has subsequently been shown to be widely distributed in endocrine tissues and their tumors (8, 9). Chromogranin A levels did not vary greatly with cAMP treatment and are shown relative to a positive control neuroblastoma cell line SKNAS (Fig. 5*B).*

FIG. 5. Western blot analysis of NSE (*A*) and chromogranin A *(B). (A)* PC3M cells were untreated (lane 1) or treated with 1 mM db-

cAMP/500 mM IBMX for 6 days. Western blot analysis was performed with antiNSE antibody. *(B)* Chromogranin A levels were examined by Western blot using antichromogranin A antibody in positive control SKNAS cells (lane 1), LNCaP cells (lanes 2 and 3), and PC3M cells (lanes 4 and 5). Cells in lanes 3 and 5 were treated for 6 days with 1 mM db-cAMP/500 µM IBMX.



Untreated LNCaP and PC3M cells expressed the panepithelial surface membrane antigen identified by the monoclonal antibody KS1/4 (10) (data not shown) and the level of KS1/4 expression was down-regulated by treatment with dbcAMP and IBMX (30% decrease in LNCaP and 74% decrease in PC3M cells).

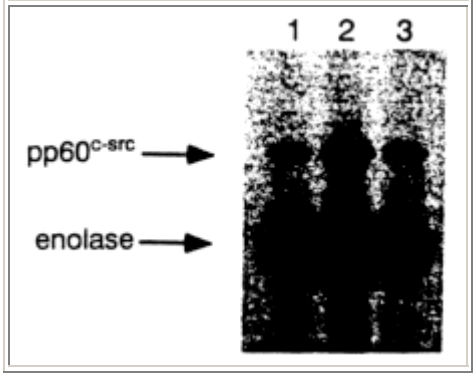
In data not shown, the intermediate filament phenotype of the cells was examined by cytochemical analysis. Both cell types were found to express cytokeratin (monoclonal antihuman epithelial keratin; Boehringer Manaheim) and low molecular weight neurofilament protein (Boehringer Mannheim), further supporting the biphenotypic nature of the cells.

LNCaP and PC3M tells were examined for expression of stagespecific embryonic antigens using anti-SSEA1, 3, and 4 antibodies and were found to express SSEA-4 (data not shown). Treatment of PC3M cells for 5 days with dbcAMP and IBMX downregulated SSEA4 expression by 75%. Although not a specific stem cell marker, SSEA expression is characteristic of embryonic cells and embryonal carcinoma cells (11). It has been reported that dbcAMP treatment induces neuronal differentiation and downregulation of SSEA expression in the teratocarcinoma cell line F9 (12). These preliminary data suggest that prostate carcinoma cells may express some stem cell markers and that these markers can be downregulated by cytodifferentiation.

Both cell lines were examined for expression of the nonreceptorlinked protein tyrosine kinase $pp60^{c\text{-}src}$, which has been reported to be a marker of neuroendocrine differentiation (13). The expression of $pp60^{c\text{-}}$

^{src} activity was low in LNCaP cells (data not shown), while pp60$^{c\text{-}src}$ activity was readily detected in PC-3M cells (Fig. 6). After treatment of PC3M cells for 6 days with dbcAMP/IBMX there was a modest increase in pp60$^{c\text{-}src}$ kinase activity as detected by autophosphorylation and phosphorylation of the exogenous substrate enolase. In contrast, treatment with TGF ß1, which is highly growth inhibitory to these cells but does not induce evidence of neuroendocrine differentiation, caused a decrease in pp60$^{c\text{-}src}$ activity. This suggests that the increase in pp60$^{c\text{-}src}$ activity with agents that elevate cAMP is related to cAMPinduced differentiation rather than growth arrest. In an experiment not shown, when cell lysates were used for both Western blot analysis and immune complex kinase assay and the radioactivity incorporated into the pp60$^{c\text{-}src}$ and enolase bands was compared to the amount of pp60$^{c\text{-}src}$ protein, it was found that cAMP treatment caused a 50% increase in the pp60$^{c\text{-}src}$ kinase specific activity, consistent with the expression of the neuroendocrine phenotype (13).

FIG. 6. *In vitro* kinase activity of pp60$^{c\text{-}src}$ in PC3-M cells. Cells were treated with vehicle only (lane 1), dbcAMP/IBMX (lane 2), or TGFß1 (10 ng/ml) (lane 3) for 6 days. Cells were immunoprecipitated with anti-pp60$^{c\text{-}src}$ antibody 327 and immunoprecipitates were incubated with [λ^{32}P]ATP and rabbit enolase. Proteins were analyzed by SDS/PAGE and ^{32}P-labeled proteins were visualized by autoradiography.



DISCUSSION

In this study, we have demonstrated that two human prostatic adenocarcinoma cell lines are multipotential neoplasias capable of epithelial and neuroendocrine differentiation. The clinical relevance of these findings is indicated by recent clinicopathologic studies showing that focal neuroendocrine differentiation is a relatively common feature of adenocarcinoma of the prostate with as many as 4796 (14) to 100% (15) of cases showing at least some evidence of focal neuroendocrine differentiation. The recent increase in the percentage of prostatic carcinomas reported to show neuroendocrine differentiation has been associated with improved methods of detection of neuroendocrine features, including the Grimelius argyrophil stain, the use of Bouin's fixative, and the use of immunocytochemistry for the detection of eutopic and ectopic hormones (16).

In an effort to understand the pathogenetic significance of neuroendocrine differentiation in prostatic carcinomas, Abrahamsson *et al* (17) studied the incidence of neuroendocrine differentiation during tumor progression by analyzing repeat biopsies in the course of disease and correlating conventional histopathologic grading of prostatic tumors with assessment of neuroendocrine cells on the basis of the Grimelius silver-staining technique and chromogramn A immunoreactivity (17). The authors found a clearly positive relationship between the degree of neuroendocrine differentiation and tumor progression, confirming their earlier observation that the more anaplastic the prostatic carcinoma, the more numerous were its neuroendocrine cells (15). As suggested by Abrahamsson *et al.*, neuroendocrine differentiation of prostatic adenocarcinoma may represent malignant transformation of a prostatic stem cell, transformation of a prostatic neuroendocrine cell, or dedifferentiation accompanied by a new program of gene expression in a transformed prostatic epithelial cell. Regardless of the cell of origin, our data suggest that although neuroendocrine features may be a poor prognostic sign if untreated or if treated with androgen ablation or cytotoxic therapy, the expression of the neuroendocrine differentiation program presents a therapeutic opportunity to induce growth arrest and terminal differentiation.

The prostate gland contains the largest number of endocrine/paracrine cells of any genitourinary organ. Neuroendocrine secretory products associated with normal prostate and prostatic carcinoma including chromogranin A, serotonin, and thyroidstimulating hormonelike and bombesinlike peptides may be valuable in screening for or in following prostatic carcinoma, especially poorly differentiated tumors that are most likely to strongly express neuroendocrine markers and that frequently have low levels of prostate-specific antigen and prostatic acid phosphatase secretion (17). In agreement with this, Kadmon *et al.* (18) recently reported that the plasma chromogranin A level was elevated in 48% of 25 patients with stage D2 prostate cancer and that in 2 patients plasma chromogranin A was the only useful blood marker for following disease progression.

It has been suggested that endocrine/paracrine cells, basal cells, and secretory cells of the prostate arise from a common precursor cell, and that multilineage differentiation is a potential of virtually all neoplasms (19). The data we have presented here demonstrate that this plasticity of prostate carcinoma cells can be observed and studied *in vitro* and that cAMP is sufficient to trigger a series of events (i.e., morphological alteration, new organelle formation, changes in enzyme synthesis and activity) associated with the emergence of the neuroendocrine phenotype. We have shown that cAMP induces these events in conjunction with growth arrest and loss of clonogenicity, consistent with the induction of terminal differendation.

Previous studies of the reversal of the transformed phenotype by cAMP have led to the proposal that cAMP can restore the integrity of normal growth regulatory pathways that have been disrupted in the process of malignant transformation (20). The critical genes regulated by cAMP in the prostate carcinoma cells could be those that induce neuroendocrine differentiation as well as those that regulate cell cycle progression. It has been shown that cAMP inhibits transcription of the growthassociated genes encoding cmyc and the transferrin receptor (21) and that cAMP blocks growth factorinduced mitogenesis by inhibition of craf-stimulated mitogenactivated protein kinase activity (22).

Metastatic prostate cancer is an incurable neoplasm. We have shown that elevation of cAMP induces terminal differentiation in human adenocarcinoma cells derived from metastatic sites. Our data suggest that the induction of terminal neuroendocrine differentiation presents an additional approach to the treatment of metastatic prostate cancer and that hydrolysisresistant cAMP analogues may be active in triggering the neuroendocrine differentiation program.

We thank Jan Endlich for scanning and transmission electron microscopy studies. We are grateful to the Developmental Studies Hybridoma Bank, maintained by The Johns Hopkins University School of Medicine and the University of Iowa, for providing monoclonal antibodies to SSEA1, 3, and 4.

1. Bang, Y.J., Kim, S.J., Danielpour, D., O'Reilly, M. A., Kim, K. Y., Myers, C. E. & Trepel, I. B. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 35563560.

2. Kozlowski, J. M., Fidler, I. J., Campbell, D., Xu, Z.L., Kaighn, M. E. & Hart, I. R. (1984) *Cancer Res.* **44**, 3S223529.

3. Cooper, M. J., Hutchins, G. M., Cohen, P. S., Helman, L. J., Mennie, R. J. & Israel, M. A. (1990) *Cell Growth Differ.* **1**, 149159.

4. Bergsagel, P. L., VictorKobrin, C., Timblin, C. R., Trepel, J. & Kuehl, W. M. (1992) *J. Immunol.* **148**, 590596.

5. Golden,A.,Nemeth,S. P. & Brugge, J. S.(1986) *Proc.Natl.Acad. Sci. USA* **83**, 85W56.

6. Shimizu, A., Suzaki, F. & Kato, K. (1983) *Biochim. Biophys. Acta* **748**, 278~284.

7. Van Eldik, L. J., Ehrenfried, B. & Jensen, R. A. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 60346038.

8. Winkler, H. & FischerColbrie, R. (1992) *Neuroscience* **49**, 497528.

9. Helman, L. J., Gazdar, A. F., Park, J. G., Cohen, P. S., Cotelingam, J. D. & Israel, M. A. (1988) *J. Clin. Invest.* **82**, 686690.

10. Simon, B., Podolsky, D. K., Moldenhauer, G., Isselbacher, K. J., Gattoni, C. S. & Brand, S. J. (1990) *Proc. Natl. Acad Sci. USA* **87**, 27552759.

11. Kannagi, R., Cochran, N. A., Ishigami F. Hakomori, S., Andrews, P. W., Knowles, B. B. & Solter, D (1983) *EMB0 J.* **2**, 23552361.

12. Andrews, P. W., Gonczol, E., Plotkin, S. A., Dignazio, M. & Oosterhuis, J. W. (1986) *Differentiation (Berlin)* **31**, 119126.

13. Brugge, J. S., Cotton, P. C., Queral, A. E., Garret, J. N., Nonner, D. & Keane, R. W. (1985) *Nature (London)* **316**, 554557.

14. di Sant'Agnese, P. A. & de Mesy Jensen, K. L. (1987) *Hum. Pathol.* **18**, 849856.

15. Abrabamsson, P.A., Wadstrom, L. B., Alumets, J., Falkmer, S. & Grimelius, L. (1987) *Pathol. Res. Pract.* **182**, 298307.

16. di Sant'Agnase, P. (1992) *Hum. Pathol.* **23**, 287296.

17. Abrahamsson, P.A., Falkmer, S., Felt, K. & Grimelius, L. (1989) *Pathol. Res.* Pract. **185**, 373380.

18. Kadmon, D., Thompson, T. C., Lynch, G. R. & Scardino, P. T. (1991) *J. Urol.* **146**, 358361.

19. Mendelsohn, G. & Maksem, J. A. (1986) in *Pathology Annual*, eds. Sommers, S. C., Rosen, P. P. & Fechner R. E. (AppletonCenturyCrofts, Norwalk, CT), Part 1, Vol. 21 pp. 91119.

20. Ashall, F., Sullivan, N. & Puck, T. T. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 39083912.

21. Trepel, J. B., Colamonici, O. R., Kelly, K., Schwab, G., Watt, R. A., Sausville, E. A., Jaffe, E. S. & Neckers, L. M. (1987) *Mol. Cell. Biol.* **7**, 2644-2648.

22. Wu, J., Dent, P., Jelinek, T., Wolfman, A., Weber, M. J. & Sturgill, T. W. (1993) *Science* **262**, 988-990.

Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 5330-5334, June 1994
Medical Sciences

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.
