[CANCER RESEARCH 48, 4770–4775, September 1, 1988]

Profiles of Prostaglandin Biosynthesis in Sixteen Established Cell Lines Derived from Human Lung, Colon, Prostate, and Ovarian Tumors[1]

Walter C. Hubbard,[2] Michael C. Alley, Theodore L. McLemore, and Michael R. Boyd

Program Development Research Group [W. C. H., M. C. A., T. L. M.] and Developmental Therapeutics Program [M. R. B.], National Cancer Institute-Frederick Cancer Research Facility, Frederick, Maryland 21701-1013

ABSTRACT

The profiles of prostanoid biosynthesis from endogenous arachidonic acid in 16 established cell lines derived from 4 histological classes of human carcinomas were determined by capillary gas chromatography-mass spectrometry. Detectable quantities of prostanoids were isolated from the culture medium of cell lines representative of the different histological classes of human tumors: colorectal adenocarcinomas (one of three cell lines); ovarian adenocarcinomas (one of three cell lines); prostate adenocarcinomas (zero of two cell lines); non-small cell carcinomas of the lung (four of five cell lines); and small cell carcinomas of the lung (zero of three cell lines). Prostaglandins E_2 and $F_{2\alpha}$ were the only prostanoids synthesized in detectable quantities. Prostaglandin E_2 biosynthesis (mean ± SD), pmol/10^6 cells, $n = 4$) in cell lines exhibiting positive prostaglandin H synthase activity was: LoVo (colorectal adenocarcinoma, 0.4 ± 0.1); A2780 (ovarian adenocarcinoma, 1.3 ± 0.3); NCI-H322 (bronchioloalveolar cell carcinoma, 8.4 ± 3.1); NCI-H358 (bronchioloalveolar cell carcinoma, 7.8 ± 2.4); EKVX (adenocarcinoma of the lung, 21.3 ± 5.5); and A427 (large cell undifferentiated carcinoma of the lung, 12.6 ± 2.8). Prostaglandin $F_{2\alpha}$ production (pmol/10^6 cells ± SD) was: LoVo (0.3 ± 0.1); NCI-H322 (0.6 ± 0.2); NCI-H358 (0.4 ± 0.1); EKVX (1.8 ± 0.4); and A427 (11.1 ± 3.1). These findings suggest that within certain limitations cultured tumor cells provide simplified experimental systems for determination of prostaglandin biosynthetic characteristics of human tumors and that prostanoid biosynthesis may be particularly characteristic of certain non-small cell carcinomas of the lung.

INTRODUCTION

There is substantial evidence that prostanoid biosynthesis may be a property of certain histological classes of human tumors (1–11). The prostaglandins and related eicosanoids synthesized from polyunsaturated fatty acid precursors have been implicated as modulators of tumor metastasis (4–8, 12–19), host immunoregulation (20–25), tumor promotion (26–36), and cell proliferation (26, 36). Since this family of compounds may mediate, in part, certain pathophysiological aspects in human malignant disease, a more complete knowledge of eicosanoid biosynthesis in human tumors is needed. A number of postulates have been forwarded for mechanisms by which eicosanoid biosynthesis may be modulated in tumor tissue. One hypothesis is that tumor cells may synthesize prostaglandins and related eicosanoids in response to certain physiological or other stimuli. Alternatively, eicosanoid biosynthesis in tumor cells may occur in response to intracellular mediators unique to malignant cells. Eicosanoid biosynthesis in tumor tissue may also occur independently of synthesis in tumor cells. Malignant cells may release mediators that specifically stimulate eicosanoid biosynthesis either in nontumorous cells resident in tumor tissue or in cells comprising the connective tissue matrix of the tumor. Given the range of disparate hypotheses forwarded for eicosanoid biosynthesis in human tumors, we sought to investigate the capacity of selected human tumor cell types to synthesize specific prostanoids from endogenous arachidonic acid. Direct comparisons of the profiles of eicosanoid biosynthesis in cultured human tumor cells make it possible, within certain limitations, to determine whether the biosynthesis of this family of compounds may be a characteristic property of the malignant cell population in certain human tumors.

In addition to providing tumor cell populations devoid of nontumorous cells which could obscure the true tumor cell biosynthetic profile, the use of cultured human tumor cells greatly reduces the number of labor-intensive procedures for sample purification typically required for the identification and quantitation of prostaglandins and related lipids in more complex cell or tissue systems. In this report, the profiles of prostaglandin biosynthesis from endogenous arachidonic acid in 16 selected cell lines derived from 4 different classes of human carcinomas are compared.

MATERIALS AND METHODS

Cell Lines. Cell lines derived from human lung, colon, prostate, and ovarian tumors were kindly provided by individual investigators as follows: NCI-H69, NCI-H322, and NCI-H358 by Drs. A. Gazdar and J. Minna (Navy Oncology Branch, National Cancer Institute); PC-3 and PC-3M by Dr. M. E. Kaighn (Laboratory of Experimental Pathology, National Cancer Institute); A2780, OVCAR4, and OVCAR8 by Drs. R. F. Ozols and T. C. Hamilton (Medicine Branch, National Cancer Institute); DMS114 and DMS187 by Drs. O. S. Pettengill and G. P. Sorenson (Dartmouth University School of Medicine); and EKVX by Dr. O. Fodstad (Norsk Hydro's Institute for Cancer Research). In addition, cell lines LoVo, DLD-1, SW-620, A427, and SK-MES-1 were obtained from American Type Culture Collection. Histological classification of these tumor cell lines is based upon previous published reports as summarized in Table 1. All cell lines were documented to be free of adventitious bacteria and pathogenic viruses and to contain only human isoenzymes.

Cell Culture. All cell lines were adapted for growth in a standard culture medium composed of RPMI 1640 (Quality Biologicals, Inc.), 10% (v/v) heat-inactivated fetal bovine serum (Sterile Systems Hyclone), and 2 mM L-glutamine (Central Medium Laboratory, National Cancer Institute-Frederick Cancer Research Facility). Cryopreserved cell stocks were maintained in liquid nitrogen vapor phase until experimental procedures were initiated. Following thaw, cells were cultivated initially for two passages in 15 ml of standard medium in T-75-cm^2 flasks at 37°C in an atmosphere of 5% CO_2-95% air at 100% relative humidity. Cell monolayers approaching 75% confluency were harvested with trypsin/EDTA (Central Medium Laboratory, National Cancer Institute-Frederick Cancer Research Facility). Suspension cell lines (NCI-H69, DMS-114, and DMS-187) were dispersed for subculture by repeated aspiration and expulsion of loosely adherent cells with a 10-ml pipet. Single cell suspensions were subcultured for a maximum of five passages in replicate T-25-cm^2 flasks (5 ml of standard culture medium) such that surface area coverage was 60 to 80% within 3 to 5 days.

Received 2/23/88; revised 5/25/88; accepted 6/3/88.

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

[1] This project has been funded at least in part with Federal funds from the Department of Health and Human Services under Contract N01-CO-74102. The content of this publication does not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the United States Government.

[2] To whom requests for reprints should be addressed, at the Program Development Research Group, Building 560, Room 32–60, Frederick, MD 21701-1013.

Table 1 *Histological classification of established cell lines*

Classification	Cell line(s)	Refs.
Colorectal adenocarcinoma	LoVo, DLD-1, SW-620	37–39
Prostate adenocarcinoma	PC-3, PC-3M	40–42
Ovarian adenocarcinoma	A2780, OVCAR4, OVCAR8	43, 44
Lung carcinomas		
Small cell carcinoma	DMS-114, DMS-187, NCI-H69	45–48
Non-small cell carcinoma	NCI-H322, NCI-H358, EKVX, A427, SK-MES-1	46, 49–52

Experimental Conditions. Cells cultured as floating aggregates were transferred to plastic centrifuge tubes (15 ml) and pelleted by centrifugation (250 × *g* for 5 min), and the standard culture medium was removed by gentle aspiration with a plastic pipet. The cells were washed twice with 5 ml of $HBSS^{+3}$ by repeated resuspension and centrifugation. The cells were resuspended in 2 ml $HBSS^{+}$. Adherent cells were prepared for experimentation by removal of standard culture medium by aspiration and two washes with 5 ml $HBSS^{+}$. The cells were then covered with 2 ml of $HBSS^{+}$. A volume of 40 μl of the medium was removed for prostaglandin analysis from all tubes and flasks prior to stimulation with the calcium ionophore A23187.

Subsequent to the addition of the calcium ionophore A23187 (5×10^{-6} M), the flasks containing adherent cells and centrifuge tubes containing cell suspensions were returned to the 5% CO_2-95% air, water-saturated atmosphere at 37°C for 15 min. Duplicate aliquots (40 μl) were then removed for determinations of prostanoid biosynthesis after stimulation with the calcium ionophore A23187.

After experimentation, the cell monolayers were washed twice with 5 ml of Hank's balanced salt solution without Ca^{2+} and Mg^{2+} and harvested with trypsin/EDTA. Total and viable cells grown in monolayer and in suspension culture were determined by hemocytometer counts in the presence of 0.4% trypan blue. Cell viability exceeded 95%. The number of cells harvested from each T-25-cm^2 flask ranged from 8.0×10^5 to 3.2×10^6. This range in cell counts reflects, in part, variations in cell size of the established cell lines.

Sample Derivatization and Extraction. Aliquots of medium for prostaglandin measurement were immediately transferred to silanized glass vials containing 0.75 to 1.25 ng each of 3,3,4,4-tetradeuterated ($^2H_4$) analogues of $PGF_{2\alpha}$, PGE_2, and $6KPGF_{1\alpha}$ as internal standards. The vial contents were dried under a nitrogen stream and the sample residue was treated in sequence with reagents for synthesis of methyloximepentafluorobenzyl estertrimethylsilyl ether derivatives as described (53). Following evaporation of excess solvents and volatile derivatization reagents under a nitrogen stream, the vial residue was extracted twice with 0.5-ml volumes of hexane (high performance liquid chromatography grade; Fisher Scientific, Fairlawn, NJ) and transferred to a clean vial. The extraction solvent was removed under a nitrogen stream and the residue containing the derivatized analytes and internal standards dissolved in 30 μl of dodecane (Aldrich Chemical Co., Milwaukee, WI) for injection into the capillary gas chromatograph.

Capillary Gas Chromatography. Volumes of 0.5 to 1.0 μl of the dodecane solution of derivatized residue were injected at 250°C in the splitless mode. The splitless mode was maintained for 0.6 min with the injector contents being split approximately 50/1 thereafter. The Finnigan MAT Model 9611 gas chromatograph (Finnigan MAT Corp., San Jose, CA) was maintained at 205°C for 1 min with subsequent temperature programming as follows: 205–245°C at 40°C/min and 245–305°C at a rate of 3°C/min. Helium (ultrahigh purity; Matheson Gas Products, Bridgeport, NJ) at a pressure head of 56 KPa (8 psi gauge) was used as the carrier gas. A 30-m SPB-5 (0.25 mm inside diameter, 0.25 μm film thickness) fused silica column (Supelco, Bellefonte, PA) interfaced to within 1.0 cm of the mass spectrometer ion source was used for vapor phase analysis.

[3] The abbreviations used are: PGH, prostaglandin H; PGH_2, prostaglandin H_2; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; $PGF_{2\alpha}$, prostaglandin $F_{2\alpha}$; TxB_2, thromboxane B_2; 9α,11β-PGF_2, 15(*S*)-9α,11β-trihydroxyprostan-5*Z*,13*E*-dien-1-oic acid; $HBSS^{+}$, Hanks' balanced salt solution containing Ca^{2+} and Mg^{2+}; $6KPGF_{1\alpha}$, 6-ketoprostaglandin $F_{1\alpha}$.

Mass Spectrometry. A Finnigan MAT 4610B mass spectrometer equipped with a SUPERINCOS data system was operated in the chemical ionization mode and programmed for the detection of negatively charged fragment ions characteristic for derivatized analytes and internal standards (53). The ions monitored were: *m/z* 524 (generated by PGD_2 and PGE_2); *m/z* 528 (fragment ion of $[^2H_4]PGE_2$); *m/z* 569 (characteristic for 9α, 11β-PGF_2 and $PGF_{2\alpha}$); *m/z* 573 (fragment ion from $[^2H_4]PGF_{2\alpha}$); *m/z* 614 (common ion generated from TxB_2 and $6KPGF_{1\alpha}$); and *m/z* 618 (derived from $[^2H_4]6KPGF_{1\alpha}$). Methane (ultrahigh purity; Matheson Gas Products), maintained at a flow rate to obtain a constant ionizer pressure reading of 0.5 torr, was used as the reagent gas. The interface oven between the capillary gas chromatograph and the mass spectrometer was maintained at 265°C. Other instrument conditions were: emission current, 0.3 mA; electron energy, 75–90 eV; and an ionizer temperature setting of 120°C. The scan rate for acquisition was 0.75 s/scan. The mass spectrometer was calibrated daily with FC-43 (Finnigan MAT) as the calibration compound. Negative ions at *m/z* 414 and *m/z* 633 were evaluated with optimization of signal intensity and peak shape.

Reagents and Standards. The $^2H_4$ analogues of PGE_2, $PGF_{2\alpha}$, and $6KPGF_{1\alpha}$, used as internal standards, were purchased from Merck Isotopes (St. Louis, MO). Unlabeled 9α,11β-PGF_2 was procured from Biomol Research Laboratories (Philadelphia, PA). The calcium ionophore A23187 and unlabeled standards of PGD_2, PGE_2, $PGF_{2\alpha}$, TxB_2, and $6KPGF_{1\alpha}$ were supplied by Sigma Chemical Co. Methoxamine HCl and pyridine (acetylation grade) used in the synthesis of methyloxime derivatives were products of Alltech/Applied Science Associates. Reagents used for synthesis of pentafluorobenzyl esters (diisopropylethylamine, pentafluorobenzyl bromide, and silylation grade acetonitrile) and trimethylsilyl esters (BSTFA and silylation grade acetonitrile) were purchased from Pierce Chemical Co.

Standard Curves. Unlabeled prostanoid standards were added to 2-ml volumes of $HBSS^{+}$ in quantities ranging from zero (blank) to 0.75 ng. The concentrations of unlabeled prostanoids present in 100-μl aliquots of $HBSS^{+}$ were determined by capillary gas chromatography-mass spectrometry as described above. 9α,11β-PGF_2 and $PGF_{2\alpha}$ were quantitated with $[^2H_4]PGF_{2\alpha}$ as the internal standard. $[^2H_4]PGE_2$ served as the internal standard for measurements of PGD_2 and PGE_2. TxB_2 and $6KPGF_{1\alpha}$ determinations were based upon $[^2H_4]6KPGF_{1\alpha}$ as the internal standard.

The presence of nondeuterated ($^2H_0$) species of prostanoids present as contaminants in the $^2H_4$ analogues used as internal standards was evaluated by determinations of $^2H_0/^2H_4$ ratios. These ratios defining the blank of each internal standard in relation to unlabeled analytes were as follows: 9α,11β-$PGF_2/[^2H_4]PGF_{2\alpha} < 0.0001$; $PGF_{2\alpha}/[^2H_4]PGF_{2\alpha} < 0.0002$; $PGD_2/[^2H_4]PGE_2 < 0.0001$; $PGE_2/[^2H_4]PGE_2 < 0.0002$; $TxB_2/[^2H_4]6KPGF_{1\alpha} < 0.0001$; $6KPGF_{1\alpha}/[^2H_4]6KPGF_{1\alpha} < 0.0002$.

Statistical Analysis. Statistical calculation of the means, standard deviations, standard errors, and level of significance are based upon the Student's *t* test.

RESULTS

Identification and Quantitation of Prostanoids. Derivatized 9α,11β-PGF_2, $PGF_{2\alpha}$, PGD_2, PGE_2, TxB_2, and $6KPGF_{1\alpha}$ are resolved with baseline separation by the vapor phase methodology as shown in Fig. 1. The identity of analyte standards is depicted above each of the representative peaks in the figure. Peak I results from the coelution of the less abundant methyloxime isomers of PGD_2 and PGE_2. The data contained in Fig. 1 differ from vapor phase analysis data contained in an earlier report (53). The differences in retention times in Fig. 1 above are shorter than those previously published and may be accounted for, in part, by a variation in the temperature programs used for vapor phase analysis. In addition, the chromatographic data contained in the figure depicts baseline resolution of derivatized 9α,11β-PGF_2 and $PGF_{2\alpha}$ not previously shown. The resolution of prostanoid analytes during vapor phase analy-

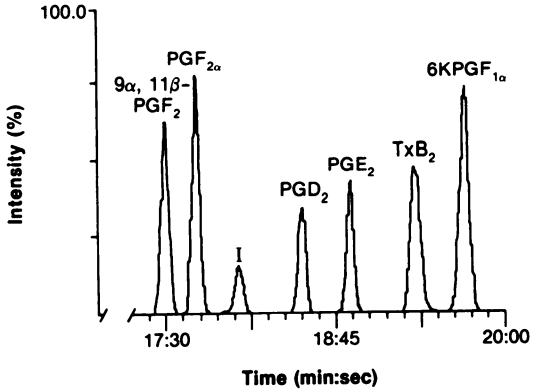

Fig. 1. Capillary gas chromatographic resolution of derivatized prostanoids. Derivatized species are as follows: $9\alpha,11\beta$-PGF_2 and $PGF_{2\alpha}$ as the pentafluorobenzyl ester-tristrimethylsilyl ether; PGD_2 and PGE_2 as the methyloxime-pentafluorobenzyl ester-bistrimethylsilyl ether; TxB_2 and $6KPGF_{1\alpha}$ as the methyloxime-pentafluorobenzyl ester-tristrimethylsilyl ether. Signals depicted are from mass spectrometric detection of negatively charged fragment ions subsequent to the injection 10 to 20 pg of derivatized analyte standards. *Ordinate*, normalized signal intensities; *abscissa*, retention times.

sis as depicted in Fig. 1 above serves, in part, as the basis for determinations of the biosynthetic profiles of prostaglandins in established cell lines derived from human tumors. The remaining criteria is the reliable discrimination between the fragment ions derived from protium and deuterium forms of prostanoids used as internal standards upon the basis of characteristic mass to charge ratios.

Standard Curves. Estimates for the detection limits per sample injection and linearity of relationships between unlabeled analytes in culture medium and internal standards were obtained subsequent to the addition of known quantities of unlabeled prostaglandins standards to 2-ml volumes of $HBSS^+$. Aliquots (100 μl) were prepared for analysis as described above, and the quantities of individual analytes found in the $HBSS^+$ were similar to quantities added. The ranges of the discrepancies in analytes added and those isolated from the buffer medium were 9–22% and include all possible sources of errors (pipeting, etc). Intraassay errors ranged from 5 to 15% and interassay errors ranged from 8 to 20%. The higher ranges in interassay and intraassay errors were confined principally to measurements using $^2H_0/^2H_4$ ratios approaching 0.001 to 0.002. These $^2H_0/^2H_4$ ratios define the limits of reliable detection and quantitation.

The 100-μl aliquots of $HBSS^+$ used in construction of the standard curves were chosen as a means of assuring that the buffer medium used in the studies did not contribute substances that could interfere with the assay. In addition, the use of 100-μl aliquots assured the transfer of quantities of analytes that would approximate $^2H_0/^2H_4$ ratios near 0.001 in samples in which 18 to 30 pg of unlabeled standards were added. Assuming that recoveries of analytes and internal standards were quantitative for the derivatization and extraction procedures, the injection of 1-μl volumes of derivatized analytes and internal standards dissolved in 30 μl of dodecane results in the detection of quantities of individual analytes ranging from 30 to 50 fg.

Profiles of Prostanoid Biosynthesis in Cultured Tumor Cells. The profiles of prostanoid biosynthesis from endogenous arachidonic acid in established cell lines derived from different histological classes of human tumors are summarized in Table 2. Prostanoids were detectable in the human tumor-derived cell lines (Table 2) representative of colorectal adenocarcinoma (1 of 3 cell lines), prostate adenocarcinoma (0 of 2 cell lines), ovarian adenocarcinoma (1 of 3 cell lines), and lung carcinoma (0 of 3 cell lines derived from small cell carcinomas; 4 of 5 cell lines derived from non-small cell carcinomas). In addition to having a higher incidence (4 of 5 cell lines) in prostaglandin biosynthesis, the cell lines originating from human non-small cell carcinomas of the lung also synthesized prostanoids from endogenous arachidonic acid in greater quantities than those derived from an ovarian adenocarcinoma and a colorectal adenocarcinoma ($P < 0.01$). The two cell lines derived from a human colorectal adenocarcinoma (LoVo) and an ovarian adenocarcinoma (A2780) synthesized less than 1.5 pmol/10^6 cells of the prostanoids whereas the four cell lines derived from human non-small cell carcinomas of the lung synthesized quantities of PGE_2 and/or $PGF_{2\alpha}$ exceeded 7.0 pmol/10^6 cells. Two of these established cell lines (NCI-H322, NCI-H358) are derived from human bronchioloalveolar cell carcinomas of the lung. The third cell line, EKVX, exhibits morphological and cytopathological features of a human lung adenocarcinoma. The fourth cell line (A427) in which prostanoid biosynthesis exceeded 7 pmol/10^6 cells of one more species of prostaglandins exhibits morphological and cytopathological features of a large cell undifferentiated carcinoma of the lung.

DISCUSSION

Earlier studies clearly show that *in vivo* production of PGE_2 is elevated in lung cancer patients with primary squamous cell carcinoma of the lung (1). Further studies of prostanoid production indicate the biosynthesis of bioreactive PGE_2 from endogenous fatty acid precursor is higher in tumor tissue, particularly in certain subclasses of lung non-small cell carcinomas, than in lung tissue from lung cancer patients (2). Extension of comparisons of prostanoid production in lung tumor tissue and in lung tissue to include the profile of $PGF_{2\alpha}$, PGD_2, TxB_2, $6KPGF_{1\alpha}$, and PGE_2 biosynthesis by chemical determination clearly shows that certain prostanoids, particularly PGE_2 and $PGF_{2\alpha}$, are released in greater quantities from tumor tissues of all subclasses of non-small carcinomas of the lung except in large cell undifferentiated carcinomas (3). The findings in each of these studies suggest that prostanoid biosynthesis may occur in tumor cells comprising pulmonary malignancies, particularly certain subclasses of non-small cell carcinomas. In studies in which the profiles of prostanoid biosynthesis in lung tumor tissue and lung tissue from lung cancer patients were compared, it is apparent that malignant cells of certain subclasses of non-small cell carcinomas of the lung may contribute to the observed differences via selective synthesis of PGE_2 and $PGF_{2\alpha}$. It is less apparent that other prostanoids may be selectively released from lung tumor cells.

The availability of cell lines derived from human solid tumors provides a simplified experimental system for investigation of eicosanoid biosynthesis in human malignancies within certain limitations. One important limitation in the use of cell lines derived from human solid tumors is the possibility that an established cell line may not adequately represent the tumor of origin.

In addition to providing cells for determination of the prostaglandin biosynthetic profiles in malignant cells, the cultured cell system facilitates the adaptation of more sophisticated analytical procedures devoid of labor-intensive steps usually required for the identification and measurement of prostaglandins and related lipids (Fig. 1). Moreover, the application of capillary gas chromatography-mass spectrometry with the inherent sensitivity of negative ion detection of electron capture derivatives makes it possible to obtain more complete profiles of prostanoid biosynthesis in cells and tissues than previously

Table 2 *Prostanoid biosynthesis in human tumor cells*[a]

Histological classification/cell line	Prostanoid (pmol/10^6 cells)					
	9α,11β-PGF_2	$PGF_{2\alpha}$	PGD_2	PGE_2	TxB_2	6KPGF$_{1\alpha}$
Colorectal adenocarcinoma						
LoVo	ND[b]	0.3 ± 0.1	ND	0.4 ± 0.1	ND	ND
DLD-1	ND	ND	ND	ND	ND	ND
SW-620	ND	ND	ND	ND	ND	ND
Prostate adenocarcinoma						
PC-3	ND	ND	ND	ND	ND	ND
PC-3M	ND	ND	ND	ND	ND	ND
Ovarian adenocarcinoma						
A2780	ND	ND	ND	1.3 ± 0.3	ND	ND
OVCAR4	ND	ND	ND	ND	ND	ND
OVCAR8	ND	ND	ND	ND	ND	ND
Lung carcinomas						
Small cell						
DMS-114	ND	ND	ND	ND	ND	ND
DMS-187	ND	ND	ND	ND	ND	ND
NCI-H69	ND	ND	ND	ND	ND	ND
Bronchioloalveolar cell						
NCI-H322	ND	0.6 ± 0.2	ND	8.4 ± 3.1	ND	ND
NCI-H358	ND	0.4 ± 0.1	ND	7.8 ± 2.4	ND	ND
Adenocarcinoma						
EKVX	ND	1.8 ± 0.4	ND	21.3 ± 5.5	ND	ND
Large cell undifferentiated carcinoma						
A427	ND	11.1 ± 3.1	ND	12.6 ± 2.8	ND	ND
Squamous cell carcinoma						
SK-MES-1	ND	ND	ND	ND	ND	ND

[a] Mean ± SD (n = 4).
[b] ND, not detectable.

obtainable by earlier mass spectrometric techniques and other more conventional analytical procedures.

Studies of arachidonic acid metabolism in established cell lines derived from human lung tumors provide further suggestive evidence that tumor cells of certain subclasses of non-small cell carcinomas of the lung selectively synthesize prostaglandins (11, 54). Eight cell lines derived from human lung tumors (two cell lines from small cell carcinomas and six cell lines from non-small cell carcinomas) used in the two groups of studies were evaluated for PGH synthase activity as determined by the cumulative levels of bisenoic prostaglandins and TxB_2 in the culture medium. All six of the established cell lines derived from non-small cell carcinomas of the lung (NCI-H322, NCI-H358, Calu-3, Calu-6, A549, and A549/Asc-1) used in these studies had demonstrable PGH synthase activities. Neither of the cell lines derived from small cell carcinomas (NCI-H69 and NCI-H128) exhibited prostanoid biosynthetic capabilities. In the present group of studies, two additional cell lines derived from human small cell carcinomas (DMS-114 and DMS-187) of the lung were also found to be devoid of PGH synthase activity. While five cell lines derived from human non-small cell carcinomas of the lung were used in the current studies, two of these cell lines (NCI-H322 and NCI-H358) had been evaluated previously for prostanoid biosynthetic capabilities (54). The major disparities in the two studies are the reported cumulative levels of synthesized prostanoids which may be related, in part, to substantial differences in the selectivity and sensitivity of the assay methods used. Four of the five cell lines derived from human non-small cell carcinomas (NCI-H322, NCI-H358, EKVX, and A427) exhibited prostanoid biosynthetic capabilities while one cell line (SK-MES-1) originating from a human non-small cell carcinoma was devoid of PGH synthase activity (Table 2). Thus, eight of nine established cell lines derived from human lung non-small cell carcinomas evaluated have demonstrable prostanoid biosynthetic capabilities while all four cell lines derived from human small cell carcinomas fail to exhibit PGH synthase activity. Similarly, the cell lines derived from other solid tumors (colorectal adenocarcinomas, prostate adenocarcinomas, and ovarian adenocarcinomas) tended to have lower incidences and lower activities of PGH synthase (Table 2).

Further comparisons of the profiles of prostanoid biosynthesis in the eight cell lines derived from non-small cell carcinomas exhibiting PGH synthase activity suggest that more than one prostanoid species may be released from the lung tumor cells. Four cell lines derived from human lung adenocarcinoma (Calu-3, Calu-6, A549, and A549/Asc-1) evaluated for PGH synthase activity convert portions of the prostaglandin endoperoxide intermediate to thromboxane A_2 (11). None of the cell lines used in the current studies exhibited thromboxane synthase activity (Table 2). PGH_2 was converted to a mixture of PGE_2 and $PGF_{2\alpha}$ in all eight cell lines representative of human non-small cell carcinomas of the lung that have been evaluated for PGH synthase activity. Two of the cell lines (Calu-3 and Calu-6) produced detectable quantities of PGD_2 while one of these cell lines (Calu-3) released detectable quantities of 9α,11β-PGF_2 and 6KPGF$_{1\alpha}$ into the culture medium (11). These findings suggest that each established cell line derived from human lung carcinomas selectively synthesize certain prostanoids.

The potential role of the prostanoids synthesized in certain subclasses of human non-small cell carcinomas of the lung have been investigated as possible modulators in several pathophysiological aspects of malignant disease (4–36). Findings in these studies suggest that the prostaglandins, thromboxane A_2, and related eicosanoids modulate, at least in part, cell proliferation, tumor metastasis, tumor promotion, and host immunoregulation. The specific role(s) of PGE_2, $PGF_{2\alpha}$, thromboxane A_2, and other prostanoids in the pathophysiology of human lung cancer, particularly in non-small cell carcinomas of the lung, await exploration.

In summary, our findings suggest that prostanoid biosyn-

thesis may be a property of certain histological subclasses of human tumors. The consistently higher rates of prostanoid biosynthesis in the cell lines derived from non-small cell carcinomas of the lung investigated herein and in previous reports suggest that this histological subclass may be of particular interest not only for further investigations of the biochemical and physiological implications of prostanoid metabolism in the etiology of specific cancers but also in the detection and possible therapeutic management of these highly lethal forms of human malignant disease.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Kathy Gill, Beverly Bales, and Sheila Testerman for preparation and organization of this manuscript for publication; and to Karen Green for technical assistance and participation in cell culture and experimental design. We wish to acknowledge the contribution of Glenn Gray for mass spectrometric analysis of the samples of biological origin.

REFERENCES

1. Seyberth, H. W., Segre, G. V., Morgan, J. L., Sweetman, B. J., Potts, J. T., Jr., and Oates, J. A. Prostaglandins as mediators of hypercalcemia associated with certain types of cancer. N. Engl. J. Med., *293:* 1278–1283, 1975.
2. Bennett, A., Carroll, M. A., Stamford, I. F., Whimster, W. F., and Williams, F. Prostaglandins and human lung carcinomas. Br. J. Cancer, *46:* 888–893, 1982.
3. McLemore, T. L., Hubbard, W. C., Litterst, C. L., Liu, M. C., Miller, S., McMahon, N. A., Eggleston, J. C., and Boyd, M. R. Profiles of prostaglandin biosynthesis in normal lung and tumor tissue from lung cancer patients. Cancer Res., *48:* 3140–3147, 1988.
4. Honn, K. V., Bockman, R. S., and Marnett, L. J. Prostaglandins and cancer: a review of tumor initiation through tumor metastasis. Prostaglandins, *21:* 833–850, 1981.
5. Rolland, P. H., Martin, P. M., Jacquemier, J., Rolland, A. M., and Toga, M. Prostaglandins in human breast cancer: evidence suggesting that an elevated prostaglandin production is a marker of metastatic potential for neoplastic cells. J. Natl. Cancer Inst., *64:* 1061–1070, 1980.
6. Bennett, A., Charlier, E. M., McDonald, E. M., Simpson, J. S., Stanford, I. F., and Zebro, T. Prostaglandins and breast cancer. Lancet, *2:* 624, 1977.
7. Bennett, A. Prostaglandins and cancer. *In*: S. M. M. Karim (ed.), Practical Applications of Prostaglandins and Their Synthesis Inhibitors, pp. 149–188. Lancaster, United Kingdom: MTP Press, 1979.
8. Bennett, A. Prostaglandins and inhibitors of their synthesis in cancer growth and spread. *In*: D. P. Rose (ed.), Endocrinology of Cancer, Vol. 3, pp. 113–126. Boca Raton, FL: CRC Press, 1982.
9. Bauknecht, T., Siegel, A., Meerpohl, H. G., and Zahradnik, H. P. Formation of prostaglandins by ovarian carcinomas. Prostaglandins, *29:* 665–672, 1985.
10. Robertson, R. P., Baylink, D. J., Marini, J. J., and Adkinson, H. W. Elevated prostaglandins and suppressed parathyroid hormone associated with hypercalcemia and renal cell carcinoma. J. Clin. Endocrinol. Metab., *41:* 164–167, 1975.
11. Hubbard, W. C., Alley, M. C., McLemore, T. L., and Boyd, M. R. Evidence for thromboxane biosynthesis in established cell lines derived from human lung adenocarcinomas. Cancer Res., *48:* 2674–2677, 1988.
12. Gasic, G. J., Gasic, T. B., and Stewart, C. C. Antimetastatic effect associated with platelet reduction. Proc. Natl. Acad. Sci. USA, *61:* 46–52, 1968.
13. Pearlstein, E., Ambrogio, C., and Karpatkin, S. Effect of antiplatelet antibody on the development of pulmonary metastasis following injection of CT26 colon adenocarcinoma Lewis lung carcinoma and B16 amelanstic melanoma tumor cells into mice. Cancer Res., *44:* 3884–3888, 1984.
14. Honn, K. V., and Sloane, B. F. Prostaglandins in tumor cell metastasis. *In*: M. Torisv and T. Yoshida (eds.), Basic Mechanisms and Clinical Treatment of Tumor Metastasis, pp. 311–333. New York: Academic Press, 1985.
15. Mehta, P. Potential role of platelets in the pathogenesis of tumor metastasis. Blood, *63:* 55–63, 1984.
16. Honn, K. V., Meyer, J., Neagos, G., Henderson, T., Westley, C., and Ratanatharathorn, V. Control of tumor growth and metastasis with prostacyclin and thromboxane synthetase inhibitors: evidence for a new antitumor and antimetastatic agent (Bay g 6575). Prog. Clin. Biol. Res., *89:* 295–331, 1982.
17. Honn, K. V., Busse, W. D., and Sloane, B. F. Prostacyclin and thromboxanes: implications for their role in tumor cell metastasis. Biochem. Pharmacol., *32:* 1–11, 1983.
18. Donati, M. B., Semeraro, N., and Gordon, S. G. Relationship between procoagulant activity and metastatic capacity of tumor cells. *In*: K. V. Honnand and B. F. Sloane (eds.), Hemostatic Mechanisms and Metastasis, pp. 84–95. The Hague: Martinus Nijhoff, 1984.
19. Mehta, P., Lawson, D., Ward, M. B., Kimurs, A., and Gee, A. Effect of human tumor cells on platelet aggregation: potential relevance to pattern of metastasis. Cancer Res., *47:* 3115–3117, 1987.
20. Sydney, E., and Salmon, M. D. Interrelationship of endogenous macrophages, prostaglandin synthesis and tumor cell clonogenicity in human tumor biopsies. *In*: T. Powles, T. J. Bockman, K. V. Honn, and P. Ramwell (eds.), Prostaglandins and Cancer: First International Conference, pp. 633–651. New York: Alan R. Liss, Inc., 1984.
21. Meerpohl, H. G., Bauknecht, T., Tritschler, V., and Lang, H. The influence of prostaglandin E_2 on macrophage mediated cytotoxicity. *In*: W. Forster (ed.), International Workshop: Heterogeneity of Mononuclear Phagocytes, pp. 428–461. New York: Academic Press, 1981.
22. Walker, C., Kristensen, F., Bettens, F., and De Weck, A. L. Lymphokine regulation of activated (G1) lymphocytes. I. Prostaglandin E_2-induced inhibition of interleukin-2 production. J. Immunol., *130:* 1770–1778, 1983.
23. Young, M. R., and Henderson, S. Enhancement in immunity of tumor bearing mice by immunization against prostaglandin E_2. Immunol. Commun., *11:* 345–352, 1982.
24. Brunda, M. J., Herberman, R. B., and Holden, H. T. Inhibition of murine natural killer cell activity by prostaglandins. J. Immunol., *124:* 2682–2688, 1980.
25. Taffet, S. M., and Russell, S. W. Macrophage-mediated tumor cell killing: regulation of expression of cytolytic activity of prostaglandin E. J. Immunol., *126:* 424–432, 1981.
26. Levine, L. Arachidonic acid transformation and tumor production. Adv. Cancer Res., *35:* 49–51, 1981.
27. Bresnick, E., Meunier, P., and Lamden, M. Epidermal prostaglandins after topical application of a tumor promoter. Cancer Lett., *7:* 121–124, 1979.
28. Verma, A., Ashendael, C. L., and Boutwell, R. K. Inhibition by prostaglandin synthesis inhibitors of the induction of epidermal ornithine decarboxylase activity, the accumulation of prostaglandins, and tumor promotion caused by 12-*O*-tetradecanoyl-phorbol-13-acetate. Cancer Res., *40:* 308–312, 1980.
29. Fischer, S. M., Mills, G. D., and Slaga, T. J. Inhibition of mouse skin tumor promotion by several inhibitors of arachidonic acid metabolism. Carcinogenesis (Lond.), *3:* 1243–1250, 1982.
30. Levine, L. Stimulation of cellular prostaglandin production by phorbol esters and growth factors and inhibition by cancer chemopreventive agents. *In*: T. J. Powles, R. S. Bockmann, K. V. Honn, and P. W. Ramwell (eds.), Prostaglandins and Related Lipids, Vol. 2, pp. 189–205. New York: Alan R. Liss, 1984.
31. Furstenberger, G., Gross, M., and Marks, F. On the role of prostaglandins in the induction of epidermal proliferation, hyperplasia and tumor promotion in mouse skin. *In*: T. J. Powles, R. S. Bockman, K. V. Honn, and P. W. Ramwell (eds.), Prostaglandins and Related Lipids, Vol. 2, pp. 239–251. New York: Alan R. Liss, 1984.
32. Fischer, S. M., and Slaga, T. J. Modulation of prostaglandin synthesis and tumor promotion. *In*: T. J. Powles, R. S. Bockmann, K. V. Honn, and P. W. Ramwell (eds.), Prostaglandins and Related Lipids, Vol. 2, pp. 255–261. New York: Alan R. Liss, 1984.
33. Fischer, S. M. Arachidonate cascade and skin tumor promotion. *In*: H. Thaler-Dao, A. Crastes de Paulet, and R. Paoletti (eds.), Icosanoids and Cancer, pp. 79–90. New York: Raven Press, 1984.
34. Furstenberger, G., Gross, M., and Marks, F. Involvement of prostaglandins in the process of skin tumor promotion. *In*: H. Thaler-Dao, A. Crastes de Paulet, and R. Paoletti (eds.), Icosanoids and Cancer, pp. 91–100. New York: Raven Press, 1984.
35. Kato, R., Nakadate, T., and Yamamoto, S. Involvement of lipoxygenase products of arachidonic acid in tumor promoting activity of TPS. *In*: H. Thaler-Dao, A. Crastes de Paulet, and R. Paoletti (eds.), Icosanoids and Cancer, pp. 101–114. New York: Raven Press, 1984.
36. Levine, L., Goldstein, S. M., Snoek, G. T., and Rigas, A. Arachidonic acid metabolism by cells in culture: Effect of tumor promoters. *In*: H. Thaler-Dao, A. Craste de Paulet, and R. Paoletti (eds.), pp. 115–121. New York: Raven Press, 1984.
37. Dexter, D. L., Barbosa, J. A., and Calabresi, P. *N,N*-Dimethyl-formamide-induced alteration of cell culture characteristics and loss of tumorigenicity in cultured human colon carcinoma cells. Cancer Res., *39:* 1020–1025, 1979.
38. Drewinko, B., Romsdahl, M. M., Yang, L. Y., Ahern, M. J., and Trujillo, J. M. Establishment of a human carcinoembryonic antigen-producing colon adenocarcinoma cell line. Cancer Res., *36:* 467–475, 1976.
39. Leibovitz, A., Stinson, J. C., McCombs, W. B., McCoy, C. E., Mazur, K. C., and Mabry, N. D. Classification of human colorectal adenocarcinoma cell lines. Cancer Res., *36:* 4562–4569, 1976.
40. Kaighn, M. E., Narayan, K. S., Ohnuki, Y., Lechner, J. F., and Jones, L. W. Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Invest. Urol., *79:* 16–23, 1979.
41. Ohnuki, Y., Marnell, M. M., Babcock, M. S., Lechner, J. F., and Kaighn, M. E. Chromosomal analysis of human prostatic adenocarcinoma cell lines. Cancer Res., *40:* 524–534, 1980.
42. Kozlowski, J. M., Fidler, I. J., Campbell, D., Xu, Z., Kaighn, M. E., and Hart, I. R. Metastatic behavior of human tumor cell lines grown in the nude mouse. Cancer Res., *44:* 3522–3529, 1984.
43. Hamilton, T. C., Young, R. C., and Ozols, R. F. Experimental model systems of ovarian cancer: applications to the design and evaluation of new treatment approaches. Semin. Oncol., *11:* 285–298, 1984.
44. Ozols, R. F. Pharmacologic reversal of drug resistance in ovarian cancer. Semin. Oncol., *12* (Suppl. 4)*:* 7–11, 1985.
45. Gazdar, A. F., Carney, D. N., Russell, E. K., Sims, H. L., Baylin, S. B., Bunn, P. A., Jr., Guccion, J. G., and Minna, J. D. Establishment of contin-

uous, clonable cultures of small-cell carcinoma of the lung which have amine precursor uptake and decarboxylation cell properties. Cancer Res., *40:* 3502–3507, 1980.

46. Brower, M., Carney, D. N., Oie, H. K., Gazdar, A. F., and Minna, J. D. Growth of cell lines and clinical specimens of human non-small cell lung cancer in a serum-free defined medium. Cancer Res., *46:* 798–806, 1986.
47. Pettengill, O. S., Sorenson, G. D., Wurster-Hill, D. H., Curphey, T. J., Noll, W. W., Cate, C. C., and Maurer, L. H. Isolation and growth characteristics of continuous cell lines from small-cell carcinoma of the lung. Cancer (Phila.), *45:* 906–918, 1980.
48. Wurster-Hill, D. H., Cannizzaro, L. A., Pettengill, O. S., Sorenson, G. D., Cate, C. C., and Maurer, L. H. Cytogenetics of small cell carcinoma of the lung. Cancer Genet. Cytogenet., *13:* 303–330, 1984.
49. Giard, D. J., Aaronson, S. A., Todaro, G. J., Arnstein, P., Kersey, J. H., Dosik, H., and Parks, W. P. *In vitro* cultivation of human tumors: establishment of cell lines derived from a series of solid tumors. J. Natl. Cancer Inst., *51:* 1417–1423, 1973.
50. Fogh, J., and Trempe, G. New human tumor cell lines. *In*: J. Fogh (ed.), Human Tumor Cells *in Vitro*, Chap. 5, pp. 115–159. New York: Plenum Publishing Corp., 1975.
51. Carney, D. N., Gazdar, A. F., Bepler, G., Guccion, J. G., Marangos, P. J., Moody, T. W., Zweig, M. H., and Minna, J. D. Establishment and identification of small cell lung cancer cell lines having classic and variant features. Cancer Res., *45:* 2913–2923, 1985.
52. Alley, M. C., Scudiero, D. A., Monks, A., Hursey, M. L., Czerwinski, M. J., Fine, D. L., Abbott, B. J., Mayo, J. G., Shoemaker, R. H., and Boyd, M. R. Feasibility of drug screening with panels of human tumor cells using a microculture tetrazolium assay. Cancer Res., *48:* 589–601, 1988.
53. Hubbard, W. C., Litterst, L., Liu, M. C., Bleecker, M. R., Eggleston, J. C., McLemore, T. L., and Boyd, M. R. Profiling of prostaglandin biosynthesis in biopsy fragments of human lung carcinomas and normal human lung by capillary gas chromatography-negative ion chemical ionization mass spectrometry. Prostaglandins, *32:* 889–906, 1986.
54. Lau, S. S., McMahon, J. B., McMenamin, M. G., Schuller, H. M., and Boyd, M. R. Differential metabolism of arachidonic acid in human lung carcinoma cell lines. Cancer Res., *47:* 3757–3762, 1987.