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

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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 prostanooids 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 E₂ and F₂α were the only prostanooids synthesized in detectable quantities. Prostaglandin E₂ biosynthesis (mean ± SD, pmol/10⁶ 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 F₂α production (pmol/10⁶ 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 prostaglandin biosynthesis may be particularly characteristic of certain non-small cell carcinomas of the lung.

INTRODUCTION

There is substantial evidence that prostanooid biosynthesis may be a propery 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 prostanooids 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. Klags (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 75-cm² flasks at 37°C in an atmosphere of 5% CO₂-95% air at 100% relative humidity. Cell monolayers approaching 75% confluency were harvested 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-cm² flasks (5 ml of standard culture medium) such that surface area coverage was 60 to 80% within 3 to 5 days.
Experimental Conditions. Cells cultured as floating aggregates were transferred to plastic centrifuge tubes (15 ml) and pelleted by centrifugation (250 x 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* 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 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 x 10^{-7} M), the flasks containing adherent cells and centrifuge tubes containing cell suspensions were returned to the 5% CO2-95% air, water-saturated atmosphere at 37°C for 15 min. Duplicate aliquots (40 µl) were then removed for determinations of prostaglandin 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 Ca2+ and Mg2+ 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%.

Subsequent to the addition of the calcium ionophore A23187, the flasks containing adherent cells and centrifuge tubes containing cell suspensions were returned to the 5% CO2-95% air, water-saturated atmosphere at 37°C for 15 min. Duplicate aliquots (40 µl) were then removed for determinations of prostaglandin biosynthesis after stimulation with the calcium ionophore A23187.

Identification and Quantitation of Prostaglandins. Derivatized prostanoid measurement were immediately transferred to silanized glass vials containing 0.75 to 1.25 ng each of 3,3,4,4-tetradeuterated (2H4) analogues of PGF2α, PGE2, and 6KPGF1α as internal standards. The vial contents were dried under a nitrogen stream and the sample residue was transferred to plastic centrifuge tubes (15 ml) and pelleted by centrifugation (250 x 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* 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 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.

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The number of cells harvested from each T-25-cm2 flask ranged from 8.0 x 10^5 to 3.2 x 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 (2H4) analogues of PGF2α, PGE2, and 6KPGF1α as internal standards. The vial contents were dried under a nitrogen stream and the sample residue was transferred to plastic centrifuge tubes (15 ml) and pelleted by centrifugation (250 x 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* 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 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.

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Identification and Quantitation of Prostaglandins. Derivatized prostanoid standards were added to 2-mi volumes of HBSS* in quantities ranging from zero (blank) to 0.75 mg. The concentrations of unlabeled prostanoids present in 100-µl aliquots of HBSS* were determined by capillary gas chromatography. The presence of nondeuterated (2H0) species of prostanoids present as contaminants in the (2H4) analogues used as internal standards was evaluated by determinations of 2H0/2H4 ratios. These ratios defining the blank of each internal standard in relation to unlabeled analytes were as follows: 9α,11β-PGF2α/[2H4]PGF2α < 0.0001; PGF2α/[2H4]PGF2α < 0.0001; 6KPGF1α/[2H4]6KPGF1α < 0.0001; PGE2/[2H4]PGE2 < 0.0001; PGD2/[2H4]PGD2 < 0.0001; TxB2/[2H4]TxB2 < 0.0001; 6KPGF1α/[2H4]6KPGF1α < 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 prostanoid standards were added to 2-mi volumes of HBSS* in quantities ranging from zero (blank) to 0.75 mg. The concentrations of unlabeled prostanoids present in 100-µl aliquots of HBSS* were determined by capillary gas chromatography.
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 prostanoid 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/10⁶ cells of the prostanoids whereas the four cell lines derived from human non-small cell carcinomas of the lung synthesized quantities of PGE₂ and/or PGF₂α exceeded 7.0 pmol/10⁶ 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/10⁶ cells of one more species of prostanoids exhibits morphological and cytopathological features of a large cell undifferentiated carcinoma of the lung.

**DISCUSSION**

Earlier studies clearly show that in vivo production of PGE₂ 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 PGE₂ from endogenous fatty acid precursor is higher in tumor tissue, particularly in certain subclasses of 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 PGF₂α, PGE₂, and PGF₂α, biosynthesis by chemical determination clearly shows that certain prostanoids, particularly PGF₂α and PGE₂, 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 PGE₂ and PGF₂α. 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 prostanoid 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 prostanoids 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
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 TxB2 in the culture medium. All six of the established cell lines derived from non-small cell carcinomas (NCI-H322, NCI-H358, Calu-3, Calu-6, A549, and A549/Asc-1) evaluated for PGH synthase enzyme. Two of these cell lines (Calu-3 and Calu-6) produced detectable quantities of PGD2 while 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 enzyme. TxB2 was converted to a mixture of PGE2 and PGF2a 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 PGD2 while one of these cell lines (Calu-3) released detectable quantities of 9α,11β-PGF2α and 6KPGF1α 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 A2, and related eicosanoids modulate, at least in part, cell proliferation, tumor metastasis, tumor promotion, and host immunoregulation. The specific role(s) of PGE2, PGF2α, thromboxane A2, 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-

### Table 2 Prostanoid biosynthesis in human tumor cells

<table>
<thead>
<tr>
<th>Histological classification/cell line</th>
<th>Prostanoid (pmol/10^6 cells)</th>
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<tr>
<td></td>
<td>9α,11β-PGF2α</td>
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<tr>
<td>Colorectal adenocarcinoma</td>
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<tr>
<td>LoVo</td>
<td>ND (ND)</td>
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<td>DLD-1</td>
<td>ND (ND)</td>
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<td>SW-620</td>
<td>ND (ND)</td>
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<td>Prostate adenocarcinoma</td>
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<tr>
<td>PC-3</td>
<td>ND (ND)</td>
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<td>PC-3M</td>
<td>ND (ND)</td>
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<tr>
<td>Ovarian adenocarcinoma</td>
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<tr>
<td>A2780</td>
<td>ND (ND)</td>
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<tr>
<td>OVCAR4</td>
<td>ND (ND)</td>
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<tr>
<td>OVCAR8</td>
<td>ND (ND)</td>
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<tr>
<td>Lung carcinomas</td>
<td></td>
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<tr>
<td>Small cell</td>
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<tr>
<td>DMS-114</td>
<td>ND (ND)</td>
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<tr>
<td>DMS-187</td>
<td>ND (ND)</td>
</tr>
<tr>
<td>NCI-H69</td>
<td>ND (ND)</td>
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<tr>
<td>Bronchioloalveolar cell</td>
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<tr>
<td>NCI-H322</td>
<td>ND (ND)</td>
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<tr>
<td>NCI-H358</td>
<td>ND (ND)</td>
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<tr>
<td>Adenocarcinoma</td>
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<tr>
<td>EKVX</td>
<td>ND (ND)</td>
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<tr>
<td>Large cell undifferentiated carcinoma</td>
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<tr>
<td>A427</td>
<td>ND (ND)</td>
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<tr>
<td>Squamous cell carcinoma</td>
<td></td>
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<tr>
<td>SK-MES-1</td>
<td>ND (ND)</td>
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</table>

* Mean ± SD (n = 4).
* ND, not detectable.
thesis may be a property of certain histological subclasses of human tumors. The consistently higher rates of prostaglandin 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.

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REFERENCES

PROSTAGLANDIN BIOSYNTHESIS IN HUMAN TUMOR CELLS

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