Peripheral Airway Cell Differentiation in Human Lung Cancer Cell Lines

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ABSTRACT

Clara cells and type II pneumocytes are the progenitor cells of the bronchioles and alveoli, respectively. These peripheral airway cells (PAC) contain characteristic cyttoplasmic structures and express surfactant associated proteins. PAC cell markers are expressed by many pulmonary adenocarcinomas having papillary and/or lepidic growth patterns, which are characteristic of the bronchioloalveolar and papillary subtypes. We investigated the expression of PAC markers in a panel of 41 lung cancer cell lines. Ultrastructural studies demonstrated the presence of cyttoplasmic structures characteristic of Clara cells or of type II pneumocytes in 9 of 34 (26%) non-small cell lung cancer cell lines, including 7 of 17 (41%) adenocarcinomas, one squamous cell carcinoma, and one large cell carcinoma. Of interest, the cyttoplasmic structures were present in 5 of 6 (83%) cell lines initiated from papillolepidic adenocarcinomas. In addition, we examined the lines for expression of the surfactant associated proteins SP-A, SP-B, and SP-C. Eight of the nine cell lines containing cyttoplasmic inclusions characteristic of PAC cells also expressed protein and/or RNA of SP-A, the major surfactant associated protein. Five of these lines expressed SP-B RNA (either constitutively or after dexamethasone induction), while a single line expressed SP-C only after dexamethasone induction. None of six small cell lung cancer cell lines examined expressed any of the PAC markers. Thus, PAC markers are expressed frequently (but not exclusively) in pulmonary adenocarcinoma cell lines, especially in those initiated from tumors having papillolepidic growth patterns. The establishment and identification of multiple cell lines expressing PAC features provide an important new resource for biological and preclinical therapeutic studies.

INTRODUCTION

The World Health Organization classification of lung cancers includes four major types (squamous cell, large cell, small cell, and adenocarcinomas) as well as several subtypes and rare tumors (1). While squamous cell carcinoma has long been regarded as the commonest type occurring worldwide, several reports have noted an apparent increase in the incidence of adenocarcinoma in some countries including the United States (2–6). The WHO classification includes only light microscopic examination. A study that combined light and ultrastructural methodologies indicated that dual adenosquamous differentiation was the commonest form of lung carcinoma (7).

The WHO classification of adenocarcinomas consists of four subtypes, including acinar (gland forming), solid, with mucin, bronchioloalveolar, and papillary (1). It is presumed that acinar carcinomas arise more centrally from bronchi, while solid adenocarcinomas represent relatively poorly differentiated tumors. BAC(2) and papillary carcinomas constitute about one-half of all pulmonary adenocarcinomas (6). Both of these subtypes have overlapping pathological features and share common histogenetic origins. BAC tumors, especially when in an invasive or metastatic mode, cannot always be differentiated from papillary carcinomas (6). The cellular origins of both of these subtypes (in humans and rodents) are heterogeneous and include mucin secreting cells, Clara cells, and type II pneumocytes (8–16). For these reasons, we prefer to regard BAC and papillary tumors as a single entity and refer to them as papillolepidic tumors, after their main histopathological features.

Because Clara and type II pneumocytes are the progenitor cells of the bronchioles and alveoli, respectively, we refer to them as peripheral airway cells. Each of these cell types contains characteristic cyttoplasmic secretory structures. The Clara cells have dense granules in the apical cytoplasm (17), while type II pneumocytes contain multimellar bodies (18). The function of the Clara cell secretions have not been determined, while the multimellar bodies of type II cells represent the storage site of surfactant.

The major function of type II pneumocytes is the production of pulmonary surfactant, which exerts a detergent-like action essential for maintaining the patency of the alveoli (for review see Ref. 19). While phospholipids comprise the major component of surfactant, several surfactant associated proteins have been identified which confer important physical properties to the phospholipids and which are necessary for function. The most abundant of these proteins is a M, 28,000–36,000 glycoprotein known as SP-A (previously referred to as SAP-35) (20–22). The cDNA for human SP-A codes for a smaller polypeptide, which undergoes extensive posttranslational modification. Recently, two other surfactant proteins, SP-B and SP-C, have been identified (23). They are M, 5,000–14,000 hydrophobic proteins and are components of active surfactant extracts utilized for the therapy of newborn infants suffering from hyaline membrane disease. The functions of Clara cells are not as well defined. They may include secretion of surfactant-like substances, water and salt transport, and mixed-function oxidation (24–26).

In this report, we describe the expression of morphological, biochemical, and molecular properties of PAC by several members of a panel of human lung carcinoma cell lines established by us. Most of the cell lines expressing PAC features were derived from adenocarcinomas, especially those having papillolepidic growth patterns.

MATERIALS AND METHODS

Cell Lines. The lung adenocarcinoma cell line A549 (27) was obtained from the American Type Culture Collection, Rockville, MD. All other cell lines were established by us. Cell lines were established from pathologically proved primary or metastatic lung carcinomas as described previously (28). Solid tumors were finely minced with scissors and dissociated into small aggregates by pipetting. Malignant effusions were collected, pelleted, washed, and resuspended in growth medium. Hemorrhagic effusions also underwent a Ficoll gradient separation procedure in order to decrease the number of RBC. Approximately 1–5 × 10⁶ cells were seeded into 25-cm² flasks. While multiple types of media were utilized, cell lines were usually initiated, established, and maintained either in RPMI 1640 supplemented with 10% heat inactivated fetal bovine serum (R10) or ACL-4, a serum-free medium we have devised for the selective culture of NSCLC and other tumors (28–30).

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1 To whom requests for reprints should be addressed.

2 The abbreviations used are: BAC, bronchioloalveolar carcinoma; PAC, peripheral airway cells; cDNA, complementary DNA; NSCLC, non-small cell lung carcinoma; SCLC, small cell lung carcinoma.
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Table 1  Expression of PAC markers in lung cancer cell lines

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>Clara cell</th>
<th>Type II</th>
<th>SP-A protein (ng/mg)</th>
<th>SP-A RNA</th>
<th>SP-B RNA</th>
<th>SP-C RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma (all)</td>
<td>4/17*</td>
<td>4/17*</td>
<td>5/15</td>
<td>7/15</td>
<td>5/15</td>
<td>1/15</td>
</tr>
<tr>
<td>BAC</td>
<td>2/2</td>
<td>0/2</td>
<td>2/2</td>
<td>2/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td>Papillary</td>
<td>2/4*</td>
<td>2/4*</td>
<td>3/4</td>
<td>3/4</td>
<td>3/4</td>
<td>1/4</td>
</tr>
<tr>
<td>Other</td>
<td>0/11</td>
<td>2/11</td>
<td>0/9</td>
<td>2/9</td>
<td>0/9</td>
<td>2/11</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>0/3</td>
<td>1/1</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
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<tr>
<td>Large cell carcinoma</td>
<td>1/5*</td>
<td>1/5*</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>0/3</td>
<td>0/3</td>
<td>0/2</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td>Mucoepidermoid carcinoma</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Carcinoid</td>
<td>0/3</td>
<td>0/3</td>
<td>0/2</td>
<td>NT°</td>
<td>NT°</td>
<td>NT°</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>0/2</td>
<td>0/2</td>
<td>0/3</td>
<td>NT°</td>
<td>NT°</td>
<td>NT°</td>
</tr>
<tr>
<td>Small cell carcinoma</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6</td>
</tr>
</tbody>
</table>

Total  5/40  6/40  5/37  8/30  5/30  1/30  9/41

* One papillary and one large cell carcinoma cell line contained both types of granules (see Table 2).
° Constitutive or dexamethasone induced expression (see Table 2).
° NT, not tested.

Table 2  Lung cancer cell lines expressing PAC cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Cytoplasmic granules</th>
<th>SP-A protein (ng/mg)</th>
<th>SP-A RNA</th>
<th>SP-B RNA</th>
<th>SP-C RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCI-H226</td>
<td>Type II</td>
<td>0</td>
<td>+</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>NCI-H322</td>
<td>Clara</td>
<td>52</td>
<td>2 +</td>
<td>C</td>
<td>Absent</td>
</tr>
<tr>
<td>NCI-H358</td>
<td>Clara</td>
<td>79</td>
<td>1 +</td>
<td>C</td>
<td>Absent</td>
</tr>
<tr>
<td>NCI-H441-4</td>
<td>Clara</td>
<td>170</td>
<td>2 +</td>
<td>C</td>
<td>1 +</td>
</tr>
<tr>
<td>NCI-H726</td>
<td>Type II</td>
<td>0</td>
<td>1 +</td>
<td>C</td>
<td>1 +</td>
</tr>
<tr>
<td>NCI-H820</td>
<td>Type II</td>
<td>460</td>
<td>2 +</td>
<td>C</td>
<td>1 +</td>
</tr>
<tr>
<td>NCI-H920</td>
<td>Type II</td>
<td>0</td>
<td>1 +</td>
<td>C</td>
<td>Absent</td>
</tr>
<tr>
<td>NCI-H1334</td>
<td>Clara</td>
<td>0</td>
<td>Absent</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>NCI-H1404</td>
<td>Clara</td>
<td>120</td>
<td>1 +</td>
<td>C</td>
<td>Absent</td>
</tr>
</tbody>
</table>

* 1+, weak expression; 2+, strong expression; C, constitutive expression; I, dexamethasone induced expression.

30). Initial cell passages were performed whenever vigorous tumor cell growth was observed. Established cell lines were passaged weekly. Anchorage independent cultures were passaged by transfer of floating multicellular aggregates. Anchorage dependent cultures were passaged at subconfluence after trypsinization. If stromal cell growth was noted in initial cultures, differential trypsinization (31) was used to obtain a pure tumor cell population. RPMI 1640, trypsin, and sera were obtained from Grand Island Biological Co., Grand Island, NY. Growth factors used in ACL-4 medium were obtained from Sigma Chemical Co., St. Louis, MO, and Collaborative Research, Bedford, MA. Cultures were maintained in humidified incubators at 37°C in an atmosphere of 5% CO₂ and 95% air.

Morphological Studies. Initial diagnostic materials (pathological and cytological) were reviewed and compared to the appearances of the corresponding cell culture and xenograft. Saccomanno fluid fixed cytoplasmic preparations of floating cultures and trypsinized adherent cultures and paraffin embedded sections of xenografts were stained with hematoxylin-eosin, Alcian blue, and mucicarmine.

For ultrastructural studies, cell pellets were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, and stained with 1% uranyl acetate, and thin sections were examined by an electron microscope.

Culture Characterization. Mycoplasma contamination was tested for by the use of a rRNA hybridization method (Gen-Probe, San Diego, CA). Cell homogenates were tested for the human forms of the following enzymes by starch gel (32) using the Authenticity system (Corning Science Products, East Walpole, MA): purine nucleoside phosphorylase (EC 2.4.2.1); glucose-6-phosphate dehydrogenase (EC 1.1.1.49); peptidase B (EC 3.4.1.14); and lactate dehydrogenase (EC 1.1.1.27). Tumorigenicity was tested for by inoculation of approximately 5 x 10⁶ cells s.c. into the flanks of male athymic nude mice of BALB/c background.

Enzyme-linked Immunosorbent Assay for SP-A. SP-A protein was measured by an enzyme-linked immunosorbent assay as described previously (33). This assay utilizes a goat anti-human SP-A IgG and a rabbit anti-human SP-A in a capture assay. Lysates from tumor cell lines were harvested and frozen at −20°C until analyzed.

Northern Blot Analyses for Surfactant Protein RNAs. RNA was extracted by the method of Chirgwin et al. (34). For Northern blot analysis, three identical gels (1.2% agarose, 7% formaldehyde) were run, loading 20 µg of total RNA per lane. After electrophoresing, the RNA was transferred to Nytran filters (Schleicher and Scimel, Keene, NH). Filters were baked at 80°C for 2 h prior to hybridization as described previously (35). Probes used for hybridization (~1 to 5 x 10⁴ cpm/ml) were partial cDNAs for SP-A (probe 7-1, a 0.9-kilobase clone); SP-B (probe 4-3, a 1.8-kilobase clone); and SP-C (probe 2-1, a 0.8-kilobase cDNA clone) (36-38). Probes were isolated from a λgt11 expression library generated from adult human lung polyadenylated RNA. The cDNA probes were labeled with [α-3²P]dCTP using a nick-translation kit.

Dexamethasone Induction of Surfactant Associated Proteins. All cell lines were tested for SP-A, SP-B, and SP-C RNA while cultured in their usual maintenance medium (R10 or ACL-4) (constitutive expression). In addition, cell lines constitutively expressing SP-A RNA were...
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![Image of tumor, culture, and xenograft morphologies of cell line NCI-H820.](image)

Fig. 1. Comparison of tumor, culture, and xenograft morphologies of cell line NCI-H820. 

**a**, malignant pleural effusion, demonstrating papillary clusters of malignant cells; 

**b**, supraclavicular lymph node from same patient, with metastatic adenocarcinoma (most of the lymph node consisted of undifferentiated metastatic tumor, with foci of papillary carcinoma (as illustrated)); 

**c**, cell line NCI-H820, initiated from lymph node metastasis, demonstrating floating clusters of tumor cells forming papillary structures; 

**d**, xenograft morphology, cell line NCI-H820, demonstrating tubulopapillary morphology.

tested for expression of SP-B and SP-C RNA after growth in the presence of dexamethasone (induced expression). Because ACL-4 medium contains hydrocortisone \(5 \times 10^{-8} \text{ M}\), cells maintained in that medium were cultured for at least 2 days in the ACL-4 medium lacking hydrocortisone and then in fresh medium lacking hydrocortisone but containing dexamethasone. Dexamethasone (Sigma) was prepared as a 10 \(\mu\text{M}\) stock in 95% ethanol and diluted to the stated concentrations in hydrocortisone-free medium.

**RESULTS**

Selection of Cell Lines. While we have initiated more than 75 NSCLC cell lines since 1977, 29 were available at the initiation of these studies in 1985. An additional 6 adenocarcinoma cell lines, initiated at a subsequent time point, also were studied. In addition to the 35 NSCLC lines, six SCLC cell lines were randomly selected from a panel of approximately 200 lines (39). All of the cell lines studied expressed human forms of the enzymes analyzed, and they were free of *Mycoplasma* contamination.

Expression of PAC Markers in Lung Cancer Lines. We examined our panel of 41 lung cancer cell lines for ultrastructural, biochemical, or molecular evidence of PAC differentiation. While not all cell lines were examined by all techniques (Table 1), all cell lines identified as containing ultrastructural evidence of PAC differentiation were examined for SP protein and RNA expression. One or more markers were present in 9 of 35 (26%) NSCLC lines (Tables 1 and 2). Multiple markers were present in 7 of 17 (41%) adenocarcinomas (including 3 of 4 papillary carcinomas and 2 of 2 BACs), 1 of 6 large cell and 1 of 3 squamous cell carcinomas. The latter cell line, NCI-H226, was initiated from a malignant pleural effusion with an original cytological diagnosis of "tumor cells, possibly mesothelioma." However, examination of the cell line demonstrated multiple biochemical and ultrastructural features of squamous cell carcinoma (40). The large cell carcinoma line (which only had ultrastructural evidence of PAC differentiation) was derived from a tumor that lacked morphological evidence of differentiation. However, the corresponding cell culture contained a gland forming subpopulation. PAC markers were not detected in carcinoids, mesotheliomas, adenosquamous, mucoepidermoid, or SCLC lines.

Properties of Cell Lines Expressing PAC Markers. The origin and properties of nine cell lines expressing one or more PAC markers are presented in Tables 2 and 3. Two cell lines were initiated from primary tumors, and seven were from metastases. They had been cultured for periods of 2 to 8 years when characterized. All but one of the patients were males, and only one patient had received therapy prior to culture. The five cultures that had been initiated in serum containing R10 medium demonstrated substrate attachment and had an epithelioid...
Fig. 2. Ultrastructural appearances of cell lines expressing PAC features. a, ultrastructural appearances of cell line NCI-H820. The cells demonstrated polarity, with microvilli being present on the apical surfaces. Many multilamellar bodies are present in the apical cytoplasm. Because of fixation artifact, the phospholipid contents of many of the bodies have been dissolved. Original magnification, ×7,050. Inset, a relatively well preserved multilamellar body. b, a multilamellar body in the process of being extruded from the apical cell surface of cell line NCI-H726. Original magnification, ×22,400.

morphism. The four adenocarcinoma cultures that had been initiated in defined ACL-4 medium (which lacks attachment factors) lacked substrate attachment. They formed papillary (Fig. 1) or inter- and intracellular gland-like structures, the lumina of which were distended with fluid, not mucin. Adenocarcinoma cells demonstrated polarity, with microvilli being present on the apical surfaces, and they were joined by tight junctions and desmosomes. All 5 cell lines tested were tumorigenic in athymic nude mice. In general, xenograft histology resembled that of the original tumor (Fig. 1). However, the xenograft histology of one BAC cell line was adenosquamous cell carcinoma.

Ultrastructural Evidence of PAC Differentiation. All 9 cell lines expressing PAC markers demonstrated cytoplasmic granules characteristic of Clara or type II pneumocytes (Fig. 2). Both of these types of granules were located in the apical cytoplasm. Clara cell granules consisted of round, oval, or irregularly shaped membrane bound structures (about 0.3 μm in diameter) with finely granular contents of variable electron density. The type II cell inclusions consisted of larger, oval membrane bound structures having whorled or lamellar osmiophilic structures. The contents of many granules appeared to have been partially or completely dissolved by fixation. On occasion, the limiting membrane of the lamellar bodies was observed to be fused with the cell membrane, and the contents were extruded into the extracellular space (Fig. 2). Clara cell and type II cell granules were each observed in three cell lines, while two cell lines contained predominantly Clara cell granules, with a subpopulation of cells containing type II cell granules.

Expression of Surfactant Proteins. SP-A RNA was detected in 8 of the 9 PAC cell lines, and in 5 of these SP-A protein was also demonstrated (Table 2; Fig. 3). SP-B RNA (constitutive or induced) was detected in 5 of the lines demonstrating SP-A RNA. In a single cell line, NCI-H820, SP-C RNA was detected. When this cell line was maintained in hydrocortisone containing ACL-4 medium, a faint band was occasionally visible on Northern blots. However, SP-C RNA could be readily detected after dexamethasone induction. In NCI-H820 cells, expression of SP-A, SP-B, and SP-C RNAs was increased after dexameth-
reportedly expressed features of type II cells (27). While multilamellar cytoplasmic structures were readily found on ultrastructural examination, neither constitutive nor dexamethasone induced expression of SP-A protein or SP-A, SP-B, or SP-C RNAs were detected.

**DISCUSSION**

We found evidence of PAC differentiation in 9 lung cancer cell lines, 7 of which were adenocarcinomas. Five of the adenocarcinoma lines were initiated from tumors that had papillolophic growth features. All of the 9 cell lines had ultrastructural characteristics of Clara cells or type II pneumocytes and 8 of them expressed SP-A RNA constitutively. In addition, five lines expressed SP-B constitutively or after dexamethasone stimulation, while a single line expressed SP-C RNA only after dexamethasone stimulation. We have previously described expression of surfactant proteins by two of the PAC cell lines described in this report, NCI-H441-4 and NCI-H820 (35, 41, 42).

Surfactant proteins were expressed by lung cancer cell lines demonstrating ultrastructural features consistent with type II pneumocytes or Clara cells. On occasion, features of both cell types were present in the same lines. These findings have been described previously in human tumors (43, 44). While Clara cells express a characteristic M, 10,000 protein (45), normal and neoplastic Clara cells also may express surfactant proteins independently of surfactant phospholipids (26, 46). Our study and others discussed above indicate the interrelationship between Clara and type II pneumocyte cells and suggest that PAC tumors arise from bronchiolar or alveolar cells capable of multipotent differentiation. In addition, PAC differentiation may, occasionally, be present in more centrally arising NSCLC tumors. We did not detect evidence of PAC differentiation in SCLC cell lines. In another study, we failed to demonstrate SP-A by immunohistochemical techniques in SCLC tumors or in adenocarcinomas of nonpulmonary origin (46).

Previous reports of established cell lines that express PAC features are very limited. A recent report in the Chinese literature describes lamellar bodies in a subline of a pulmonary carcinoma culture, but not in the parent cell line (47). Multilamellar bodies have also been described in a cell line established from the lung of a sheep with jaagsiekte (ovine pulmonary adenomatosis) (48). Jaagsiekte is a virally induced ovine disease characterized by hyperplastic or tumorous proliferation of type II pneumocytes. We also studied A549 cells, a human lung carcinoma cell line widely believed to be of BAC origin. The A549 line was described by Giard and associates in 1973 as being initiated from a tumor designated simply as “lung carcinoma” (49). Xenografts of the cell line contained acinar structures. In 1976 Lieber et al. (27) described ultrastructural features and phospholipid formation characteristic of type II pneumocytes in A549 cells and claimed that the line was initiated from an “alveolar cell carcinoma.” However, their illustration (and description) of the tumor histology is that of a gland forming acinar carcinoma with mucin formation. Other published reports have suggested that A549 cells do not express biochemical properties characteristic of type II cells (50, 51). In addition, TGF-β induces goblet cell differentiation in A549 cells (52). While our studies confirmed previous reports that A549 cells contained cytoplasmic lamellar structures (27), the cells failed to express surfactant protein or RNAs. The ultrastructural appearances of Clara and type II granules are not completely diagnostic, and other cell types may contain artifac-

**Fig. 3.** Northern blots, demonstrating constitutive expression of SP-A and SP-B RNA by lung cancer cell lines. Top, SP-A RNA (2.20-kilobase band) expressed by cell lines NCI-H920 (adenocarcinoma), NCI-H441 (BAC), NCI-H358 (BAC), and NCI-H226 (squamous cell carcinoma); bottom, fainter 2.0-kilobase (kb) band, representing SP-B expression, present in cell lines NCI-H920 and NCI-H441.

**Fig. 4.** Northern blots of cell line NCI-H820, derived from a papillary adenocarcinoma, demonstrating dose dependent dexamethasone induction of surfactant protein RNAs SP-A, SP-B, and SP-C. The cell line was cultured in steroid free medium for 48 h and then exposed to varying concentrations of dexamethasone for 4 h. kb, kilobases.
tual or real inclusions with similar morphologies (53, 54). Thus, we regard only those cell lines that express both ultrastructural and molecular or biochemical markers as having unequivocal evidence of PAC differentiation. Using this criterion, eight of our cell lines appear to be the first continuous lines of any species that demonstrate definite evidence of PAC differentiation.

While most of our cell lines expressing PAC features were initiated from papillolepidic tumors, these tumors form a heterogeneous group, as discussed previously. In addition, some other NSCLC lung tumors may express markers of PAC differentiation (43, 50, 55). Also, metastases from non-lung carcinomas may have papillolepidic growth features (44, 56, 57). Thus, papillolepidic morphology and expression of PAC markers are suggestive, but not diagnostic, of PAC histogenesis.

Our PAC cell lines may be useful for several types of studies including: (a) the physiology of surfactant and its proteins (41, 42); (b) the biology of Clara cells and their role in xenobiotic metabolism; (c) in vitro testing of new therapeutic modalities specifically directed at PAC cells, such as 4-ipomoxalin (58); and (d) preparation of human-derived materials potentially useful for the treatment of the respiratory distress syndrome of the newborn and other surfactant deficient states (21, 59). The establishment of multiple human cell lines manifesting unequivocal evidence of PAC differentiation provides a major new resource for biological and preclinical therapeutic studies.

REFERENCES


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