Cytoskeleton-associated Proteins of Human Lung Cancer Cells

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ABSTRACT

The proteins associated with the cytoskeletal framework of the major forms of lung cancer in culture were analyzed by twodimensional gel electrophoresis of cytoskeletal framework (CSK) fractions. We have found that (a) small-cell carcinoma (SCC) lines, which differ from non-SCC lung carcinomas (adenocarcinoma, squamous carcinoma, and large-cell undifferentiated carcinoma) in morphology and growth characteristics, had a CSK composition different from that of non-SCC lines. Six separate SCC lines contained protein 1 (M, 140,000, pi 5.2), protein 2 (M, 140,000, pi 5.1), protein 3 (M, 190,000, pi 7.6), and protein 4 (M, 190,000, pi 7.5) but lacked protein 5 (M, 80,000, pi 6.1), protein 6 (M, 48,000, pi 5.6), and protein 7 (M, 55,000, pi 5.5); four non-SCC lines lacked proteins 1 to 4 but contained proteins 5 to 7. Protein 7 was identified to be vimentin. (b) SCC lines, which have neuroendocrine properties, contained CSK proteins characteristic of neuroblastoma cells. Proteins 1 to 4 were found in all SCC lines, in two neuroblastoma lines, but not in other human cell lines. (c) SCC and non-SCC lines contained protein 8 (M, 40,000, pi 5.4) and protein 9 (M, 48,000 to 49,000, pi 6.4), which comigrated with purified keratin. These proteins were also detected in other carcinomas but not in neuroblastoma, leukemia, and fibroblast cell lines. (d) Carcinomas of the lung were distinguishable from other carcinomas by their CSK components. Nonpulmonary carcinomas lacked proteins 1 to 4 which were found in SCC lines and also lacked proteins 5 and 6 found in non-SCC lung cancer cells. The differences in the content of CSK proteins between cell lines were not due to differences in the distribution of a given protein between CSK, soluble, and nuclear fractions. These studies may have biological and clinical importance since SCC has greater metastatic capacity and therapeutic responsiveness than non-SCC lung cancers. In addition, the identification of these CSK proteins by immunohistochemical staining in human lung cancers and normal bronchial epithelium could help clarify the lineage relationships between normal bronchial epithelial cells and the different forms of lung cancer and lead to more precise diagnostic classification of lung cancer biopsy specimens.

INTRODUCTION

It has been well recognized that SCC of the lung is a clinical and pathological entity distinguishable from the non-small cell lung cancers [squamous carcinoma, adenocarcinoma, and large cell undifferentiated carcinoma (10)]. SCC, composed of small cells with a high ratio of nucleus to cytoplasm and finely granular nuclear chromatin (22), has an extreme propensity to metastasize early and to multiple distant sites (7). It is also frequently associated with ectopic hormone secretion (4) and is one of the few solid tumors in adults that are highly responsive to chemotherapy and radiotherapy (17).

Cell lines derived from SCC are also distinguished by their morphological and functional characteristics. SCC lines, unlike non-SCC lines, often grow as floating aggregates or spheroids, have neurosecretory granules, frequently secrete polypeptide hormones, such as adrenocorticotropic hormone and antidiuretic hormone, and contain high levels of 3,4-dihydroxy-L-phenylalanine decarboxylase (1, 14, 16). The finding of these neuroendocrine properties led to the presumption that SSCs arise from the normal amine precursor uptake and decarboxylating cells in the respiratory tract (25). However, there is increasing evidence that the neuroendocrine features of SCC overlap with the other major types of lung cancer (2). There is a need, therefore, to better define the lineage relationships between the different forms of lung cancer. It is also important to identify the cellular components of SCC that may be responsible for its characteristic morphology and metastatic capacity. In a recent study, we determined that the surface protein phenotype of cultured SCC is distinct from that of the other major forms of human lung cancer in culture (3). We now report that SCC also contains cytoskeleton-associated proteins that are different from those of non-SCC lung cancer lines.

MATERIALS AND METHODS

Cell Lines. All the cell lines were grown in Roswell Park Memorial Institute Tissue Culture Medium 1640 with 10% fetal calf serum and 1 mm glutamine. The growth characteristics of the various lung cancer lines [OH-1, HUT 69, HUT 60, HUT 64, HUT 128, HUT 231, HUT 23, HUT 125, HUT 157 (1), and U1752 (4)] were described previously. The neuroblastoma cell lines (CHP-100 and CHP-212) were supplied by Dr. A. Schlesinger (28). The leukemia cell lines (HL60, U937, and CEM) were gifts of Dr. H. Lazarus. MCF-7 and FS-2 were provided by Lan Bo Chen. CCL 228, CRL 1420, and CRL 1550 were obtained from the American Type Culture Collection, Rockville, Md.

Labeling of Cellular Proteins with [35S]Methionine. Cells were washed with PBS 3 times and then incubated in methionine-deficient Roswell Park Memorial Institute Tissue Culture Medium 1640 with 10% dialyzed fetal calf serum and 1 mm glutamine. [35S]Methionine (New England Nuclear, Boston, Mass.) was added at 100 µCi/ml. After 3 hr of incubation, the suspension cell lines were transferred to centrifuge tubes and washed 3 times with cold PBS; attached cell lines were scraped from the culture dish, transferred to centrifuge tubes, and washed 3 times with cold PBS. Approximately 1 × 10⁶ trypan blue-excluding cells were used for each CSK extraction.

CSK Extraction and Resolution by 2-Dimensional Gel Electrophoresis. The cells were suspended in a SD buffer which contains 1% Triton X-100, 10 mm 1,4-piperazinediethanesulfonic acid, 100 mm KCl, 300 mm sucrose, 2.3 mm MgCl₂, 1 mm phenylmethylsulfonyl fluoride (added immediately before use), and 100 kallikrein inhibitor units of aprotinin per...
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RESULTS

SCC. OH-1 cells have the morphology and growth characteristics of SCCs in culture (1, 14). The cells are round or oval shaped and slightly larger than human lymphocytes. The nucleus is dense; the cytoplasm is scant. OH-1 cells grow as aggregates in suspension, and as the aggregates enlarge, spheroids with a central area of necrosis are formed (21). For the present study, we used exponentially growing cells which were harvested and extracted prior to the formation of necrotic centers. Another SCC cell line, NCI H69 (14), has a slightly different morphology from OH-1. The cells are somewhat larger and grow as loose aggregates in suspension. The 2-dimensional gel pattern of CSK extracts of OH-1, shown in Fig. 1a (pI 5 to 7) and Fig. 1b (pH 7 to 9), is very similar to that of NCI-H69 (Fig. 1, c and d). Most notable is the presence of 4 proteins, P1, P2 (Fig. 1, a and c), and P3 and P4 (Fig. 1, b and d), present in the CSK fractions of OH-1 and NCI-H69 which cannot be detected in the CSK of non-SCC lung cancers (Figs. 2 and 3). Conversely, OH-1 and NCI-H69 lack 3 CSK proteins, P5, P6, and P7, which are present in the CSK of non-SCC lung cancer lines. Several CSK proteins are found in both SCC and non-SCC cell lines [P8 and P9 (Fig. 1, a and c) and P10, P11, P12, and P13 (Fig. 1, b and d)]. Protein A (M, 43,000, pI 6.1) comigrates with α-, β-, and γ-actin and is present in all the human cell lines (Figs. 1 to 5). Aside from actin, the other CSK proteins we have described are not present in the soluble fraction (Fig. 5).

We have also analyzed the CSK extracts from 4 other SCC cell lines (NCI-H60, NCI-H64, NCI-H128, and NCI-H231) and found them to contain the same protein patterns as OH-1 and NCI-H69 (Table 1).

Non-SCCs. The cell lines NCI-H23 and NCI-H125 have been characterized as human lung adenocarcinomas (14). Both cell lines are composed of flat polygonal cells that are tightly adherent to the plastic dish. The cytoplasm is highly granular, similar to nonpulmonary adenocarcinomas in culture. U1752 is a cell line of human lung squamous carcinoma cells (4). The majority of U1752 cells are flat and polygonal, but a few spindle-shaped cells with cytoplasmic processes were noted. NCI-H157 is derived from a human large-cell undifferentiated carcinoma (14). The cells are oval or elongated and are tightly adherent to the plastic dish. A few cells are large and flat with several large nuclei and prominent nucleoli, resembling the giant multinucleated cells found in tissue sections of human large-cell undifferentiated lung tumors. Tumors formed by implantation of these different cell lines in nude mice had a similar histological pattern to the original tumors (14).

The 2-dimensional gel patterns of the CSK extracts from these non-SCC cell lines, NCI-H23 (Fig. 2, a and b), NCI-H125 (Fig. 2, c and d), U1752 (Fig. 3, a and b), and NCI-H157 (Fig. 3, c and d), are very similar. They all lack P1 to P4 and contain P5, P6, and P7, which distinguish them from SCC. The presence of P5 and P6 also distinguishes the non-SCC cell lines from nonpulmonary carcinomas (Table 1). P7 comigrates with vimentin purified from a hamster cell line, BHK-21. Associated with P7 is a cascade of protein spots, indicated by unnumbered brackets, that are known to be degradation products of vimentin (13). The cascade of proteins appears in spite of the presence of the proteolytic inhibitors aprotinin and phenylmethylsulfonyl fluoride in the CSK extraction buffer. Incubation of 35S-labeled CSK extract with unlabeled total cell lysate of SCC or non-SCC cells

Table 1

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<th>Protein</th>
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<td>48-49</td>
<td>7.5</td>
</tr>
<tr>
<td>P13</td>
<td>62</td>
<td>6.4</td>
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Lung SCC lines
OH-1, NCI-H69, NCI-H60, NCI-H128, and NCI-H231
Lung adenocarcinoma lines NCI-H23 and NCI-H125
Lung squamous carcinoma lines U1752
Lung large cell undifferentiated carcinoma lines NCI-H157
Neuroblastoma lines CHP-100 and CHP-212
MCF-7 (breast), CCL228 (colonic), CRL1420 (pancreatic), and CRL-1550 (cervical carcinomas)
FS2 fibroblast (foreskin)
Poorly differentiated leukemia lines HL60 (myeloid) and U937 (monocytoid)
does not increase the amount of vimentin-associated proteins or induce any of the CSK proteins (P1 to P13). Therefore, the CSK proteins that we describe, including the vimentin-associated proteins, are unlikely to be products of proteolysis during the CSK extraction procedure.

Like SCC and the nonpulmonary carcinomas, non-SCC cells contain P8 and P9, groups of proteins that comigrate with keratin from cultured human keratinocytes. One of the P9 proteins found in SCC has a slightly higher molecular weight than those found in the non-SCC and other carcinomas. We have confirmed the presence of keratin in SCC, non-SCC, and other human carcinomas by immunofluorescence and immunoblotting with anti-keratin antibody (data not shown). Similar to SCC and nonpulmonary carcinomas, the non-SCC cell lines contain P10, P11, P12, and P13 in their CSK fractions but not in their soluble fractions.

Neuroblastoma. CHP-100 and CHP-212 have been characterized as human neuroblastoma cell lines (14). These cells are small with a large nucleus, scanty cytoplasm, and short processes. The CSK extracts of these cells, analyzed by 2-dimensional gel electrophoresis, are shown in Fig. 4. Most notable is the presence of 4 proteins that comigrate with SCC proteins (P1, P2, P3, and P4). None of the other cell lines we have tested so far except the SCC lines contains these proteins. Although P1 to P4 have high molecular weights (M, 150,000 to 200,000) like neurofilament proteins, they do not have pl5 characteristic of neurofilament subunits (35). Neuroblastoma CSK, like SCC CSK, lacks P5 and P6. Unlike SCC CSK, neuroblastoma CSK contains P7 (vimentin) and lacks P8 and P9. Similar to the other human cell lines we tested, neuroblastoma CSK contains P10, P11, P12, and P13.

Nonpulmonary Carcinomas, Fibroblasts, and Leukemias. The CSK extracts of nonpulmonary carcinomas, MCF-7 (breast carcinoma), CC1 228 (colon carcinoma), CRL 1420 (pancreatic carcinoma), and CRL 1550 (cervical carcinoma) all contain P8 and P9 (keratin), similar to SCC and non-SCC cell lines (Table 1). The nonpulmonary carcinomas contain P7 (vimentin), P10, P11, P12, and P13. They do not contain P1 to P4 which distinguishes them from SCC and neuroblastoma. They also do not contain P5 and P6, which distinguishes them from non-SCC lung cancer lines.

FS-2 (human foreskin fibroblast line) and the leukemias HL60 and U937 do not contain P8 and P9 (keratin) and P1 to P6. P7 (vimentin) is present in FS-2 but is absent in the poorly differentiated leukemia cells HL60 and U937.

Soluble and CSK plus Nuclear Fractions. The proteins P1 to P13 are not detected in the soluble fractions of the different cell lines. The soluble fractions of OH-1 and NCI-H25 are shown in Fig. 5, a to d as examples. The M, 60,000 protein bands with pl 5.2 have electrophoretic mobilities consistent with those of tubulins, which are known to be solubilized during the detergent extraction (20).

The CSK plus nuclear fractions of the different cell lines were examined and compared with the CSK fractions. The proteins (P1 to P13), when absent from the CSK of a particular cell line, did not appear in the CSK plus nuclear fraction. P5, P6, and P7, which were not detected in the CSK of OH-1 cells, were also not detected in the CSK plus nuclear fraction (Fig. 6a). Likewise, P1 and P2 which were not present in the CSK of NCI-H25 cells were not present in the CSK and nuclear fraction of these cells (Fig. 6b).

DISCUSSION

The cytoskeleton appears to have a role in cell shape, cell motility, anchorage-dependent growth, distribution of cell surface proteins, and the localization of organelles such as mitochondria and polyribosomes (8, 9, 18–20, 23, 26). The functional complexity of the cytoskeleton is matched by its structural complexity: extensive interconnecting networks that include microfilaments; microtubules; the intermediate filaments; and the highly cross-linked microtubular lattice (26). Many of the proteins that compose the major CSK filaments have been characterized. Among these proteins are actin in microfilaments, tubulin in microtubules, and the heterogeneous group of intermediate filament proteins that are associated with different cell types [keratin in epithelial cells, vimentin in fibroblasts, desmin in muscle cells, glial filament protein in glial cells, and the neurofilament protein subunits in neural tissue (20, 28, 35)]. In addition, many other proteins have been described to be components of the cytoskeleton (11, 15, 20, 29, 31).

In the present study, we analyzed the CSK framework proteins obtained after double-detergent extraction of the major morphological types of human lung cancer cell lines (SCC, adenocarcinomas, squamous carcinoma, and large-cell undifferentiated carcinoma). The SD buffer solubilizes membranes, lipids, and three-fourths of cytoplasmic proteins (soluble fraction), leaving the major filament networks and nucleus intact (2). The DD buffer solubilizes the filament network; the nuclei can then be pelleted by centrifugation. We have defined the CSK proteins as those that remained insoluble after SD buffer extraction but were solubilized by DD buffer. The CSK fraction, however, does not contain all the CSK proteins. Tubulin, for example, appears in the soluble fraction (20). Some of the intermediate filaments may remain with the nuclear pellet after DD buffer extraction (6, 30). The proteins (P1 to P13) that we describe in this study were present as major components in the CSK fraction but were not detected in the soluble fraction. When absent from the CSK fraction of a cell line, the proteins (P1 to P13) did not appear in the CSK plus nuclear fraction of that cell line. Thus, although the fractions we analyzed were empirically defined, the differences in the content of P1 to P13 between cell lines were not due to differences in the distribution of a given protein between soluble, CSK, and nuclear fractions. Our primary aims were to determine whether each of the morphological types of human lung cancer is associated with a specific set of CSK proteins and whether CSK protein expression reflects the differentiation properties of the different types of human lung cancer. This indeed, appears to be the case based on several findings. (a) We have found that SCC, a tumor characterized by its neuroendocrine features, possesses the CSK proteins (P1 to P4) which are characteristic of neuroblastoma cells. The precise nature of these shared proteins is not known. Although the molecular weights of P1 to P4 are similar to those of neurofilament protein subunits isolated from calf brain (M, 20,000 to 150,000), their isolectric focusing mobilities do not correspond to those of neurofilament proteins described previously (35). (b) Non-SCC lung cancer lines contain P5 to P7 but not P1 to P4. Thus, the CSK pattern is distinct from that of non-SCC lung tumor cells. In our study of surface proteins, we found that a common pattern existed for the types of non-SCC lung cancer cells (3). We now find that these non-SCC lung cancer cells also share a common set of CSK proteins. These biochemical findings appear to be consistent with propos-
als that adenoc- and squamous lung carcinomas, in particular, arise from a common pool of precursor cells (2). (c) Carcinomas of the lung were distinguishable from other carcinomas by their CSK components. Nonpulmonary carcinomas lacked P1 to P4 found in SCC cells and P5 and P6 present in non-SCC lung carcinomas. Thus, carcinoma cells derived from different tissues may also be distinguished by their unique sets of CSK proteins. Antibodies specific for these different proteins may be helpful in distinguishing primary lung cancers from other cancers metastatic to the lung.

The expression of some of the CSK proteins noted in this study seems to correlate with the morphology and adherence characteristics of lung cancer cells in vitro. P7 (M, 55,000, pl 5.5) comigrates with purified vimentin isolated from the human fibroblast line, FS2. P7 is associated with a cascade of proteins (indicated by unnumbered brackets) that are similar to the degradation products of vimentin in electrophoretic mobility (13). We have noted in this study that non-SCC lung cancer lines (which are flat and adherent to the culture dish like nonpulmonary carcinomas, neuroblastoma, and fibroblasts) contain large amounts of P7, detectable by 2-dimensional gel electrophoresis. We have also confirmed the presence of vimentin in non-SCC lung cancer lines by immunofluorescence with anti-vimentin antibody. 4 It is possible that non-SCC lines express vimentin, a mesenchymal-type intermediate filament protein, under culture conditions that allow cellular attachment and spreading. Similar observations of vimentin expression during in vitro cultivation of epithelial cell lines have been reported (19, 32). Interestingly, we have noted previously that large amounts of vimentin are synthesized during differentiation of HL60 and U937 into macrophage-like cells that flatten and adhere to the substrate (5). It is probable, therefore, that vimentin expression influences cell morphology and adherence characteristics in vitro. SCC lines, similar to poorly differentiated leukemia cell lines, grow in suspension and contain little or no P7. These cell lines also do not contain the cascade of proteins that are presumed to be degradation products of vimentin. We are now investigating whether other modified forms of vimentin, such as phosphorylated variants that differ from P7 in electrophoretic mobility, can be induced in SCC lines.

SCC and non-SCC lung cancer lines contain P8 (M, 41,000, pl 5.4) and P9 (M, 48,000 to 49,000, pl 6.4) which comigrate with keratin from cultured human epidermal cells. These proteins were also present in nonpulmonary carcinomas but not in neuroblastoma, fibroblasts, and leukemias. We have confirmed the presence of keratin in SCC and non-SCC lines by immunofluorescence with antikeratin antibody (data not shown). One of the P9 proteins identified in SCC lines has a higher molecular weight than those found in non-SCC lung cancer lines. Further characterization of these proteins may help clarify the lineage relationships between SCC and non-SCC lung cancers, since there is evidence to suggest that distinct sets of keratin genes are expressed during epithelial cell differentiation in different epithelial tissues and in different developmental stages (12, 27, 33, 34).

Several major proteins specifically associated with the cytoskeleton are ubiquitous in human cell lines. P11, P12, and P13 are present in the CSK fraction of all the human cell lines we tested; P10 is detectable in all human cell lines except the leukemias.

It is apparent from our studies that human lung cancer lines which exhibit differences in morphology, adherence characteristics, and differentiation properties also express different CSK proteins. The major forms of lung cancer may represent a good model system for correlating the expression of subsets of CSK proteins with structural and functional characteristics of neoplastic cells. The knowledge gained from such studies should have both biological and clinical importance, since SCC has greater metastatic capacity and responsiveness to therapy than non-SCC lung cancers. The CSK proteins we have described clearly separate SCC and non-SCC lung cancer cells. The identification of these different CSK proteins in human lung cancers and normal bronchial epithelium might help define the cell lineages of the different forms of lung cancer and the pathways of epithelial cellular differentiation in the bronchial mucosa.

ACKNOWLEDGMENTS

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REFERENCES


Fig. 1. CSK proteins of small-cell carcinoma lines OH-1 (a and b) and NCI-H69 (c and d). A, protein A.
Fig. 2. CSK proteins of adenocarcinoma lines NCI-H23 (a and b) and NCI-H125 (c and d). Unnumbered brackets, enclosure of a cascade of protein spots associated with P7; A, protein A.
Fig. 3. CSK proteins of squamous carcinoma line U1752 (a and b) and large-cell undifferentiated carcinoma NCI-H157 (c and d). Unnumbered brackets, enclosure of a cascade of protein spots associated with P7; A, protein A.
Fig. 4. CSK proteins of neuroblastoma cell lines CHP100 (a and b) and CHP212 (c and d). Unnumbered brackets, enclosure of a cascade of protein spots associated with P7; A, protein A.
Fig. 5. Soluble proteins of small-cell carcinoma OH-1 (a and b) and adenocarcinoma NCI-H23 (c and d). A, protein A.
Fig. 6. CSK plus nuclear fractions of small-cell carcinoma OH-1 (a) and adenocarcinoma NCI-H23 (b). Unnumbered brackets, enclosure of a cascade of protein spots associated with P7; A, protein A.
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