IA-1, a New Marker for Neuroendocrine Differentiation in Human Lung Cancer Cell Lines

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Abstract

IA-1 is a recently isolated novel complementary DNA which encodes a protein of 510 amino acids that contains both a zinc finger DNA-binding domain and a putative prohormone domain. mRNA expression of IA-1 has been found thus far only in tumors of neuroendocrine origin. In this report we describe the expression of IA-1 mRNA in a panel of 64 human lung cancer cell lines. IA-1 mRNA was detected by Northern blot analysis in 97% (30 of 31) of small cell lung cancer cell lines. In contrast, IA-1 mRNA was detected in only 13% (4 of 30) of non-small cell lung cancer cell lines. Nine of the 30 (30%) expressed either chromogranin A mRNA or produced L-dopa decarboxylase. Four of these 9 (44%) had detectable levels of IA-1 mRNA. In most of the lung cancer cell lines examined, IA-1 showed high concordance with the other neuroendocrine markers, L-dopa decarboxylase, and chromogranin A. The one exception was a variant small cell lung cancer cell line which expressed low or nondetectable levels of L-dopa decarboxylase. IA-1 is a candidate marker of neuroendocrine differentiation of human lung tumors.

Introduction

Lung cancer is the most common cause of cancer deaths in the United States (1). About 20% of the cases are classified as SCLC (2, 3). The other 80% consist of squamous cell carcinomas, large cell carcinomas, and adenocarcinomas, and are referred to as NSCLC. Lung cancer cell lines have been established which grow in specifically defined hormone-supplemented medium and in many cases these cell lines retain the morphology, cytology, and histological appearance of the original biopsy specimens (4-8). Many SCLC cell lines have amine precursor uptake and decarboxylation cell properties. These lines have increased sensitivity to both cytotoxic drugs and radiation therapy when compared to NSCLC cell lines (9). Neuroendocrine phenotypes of SCLC are demonstrated by expression of multiple markers of neuroendocrine differentiation such as presence of dense core granules by electron microscope, DDC enzyme activity, chromogranin A, neurospecific enolase, neural cell adhesion molecule, Leu-7, gastrin-releasing peptide, and synaptophysin (10). Some NSCLCs express neuroendocrine markers, and those expressing two or more markers have shown a greater likelihood of responding to chemotherapy (11-13). Neuroendocrine markers are currently used for detecting neuroendocrine differentiation in lung cancer cell lines, and new markers with greater sensitivity and specificity may help define a subset of patients with NSCLC who are more likely to benefit from chemotherapy.

Recently, we isolated a novel cDNA, IA-1, from human insulinoma tissue (14). IA-1 is expressed as a single 3.0-kilobase transcript encoding a protein of 510 amino acids which consists of a putative prohormone sequence at the NH₂-terminal domain and a five zinc finger DNA-binding motifs at the COOH-terminal domain. Northern analysis showed that IA-1 is expressed in tumors of neuroendocrine origin, including pheochromocytoma, medullary thyroid carcinoma, insulinoma, pituitary tumor, and small cell lung carcinoma, but not in a variety of other tumors such as melanoma, ovarian carcinoma, breast carcinoma, thyroid carcinoma, glioblastoma, and choriocarcinoma or in normal tissues including pancreas, testes, lymph node, brain, lung, liver, stomach, spleen, thyroid, pituitary, kidney, and colon (14).

In this study, we examined the expression of IA-1 in 64 endocrine and nonendocrine human lung cancer cell lines and compared it with two other neuroendocrine markers, chromogranin A and L-dopa decarboxylase (15, 16). DDC is the key enzyme in the amine precursor uptake and decarboxylation cell system and was shown to be a useful marker for identifying tumors of neuroendocrine cell lineage. Chromogranin A is the major matrix substance in dense core secretory granules which represent the cytoplasmic storage sites of specific peptide and amine products of neuroendocrine cells.

Materials and Methods

Lung Cancer Cell Lines. Human SCLC and NSCLC cell lines were established, maintained, and characterized at the National Cancer Institute-Navy Medical Oncology Branch as described previously (6, 9). Classic SCLC grows as tightly packed floating cellular aggregates, and expresses elevated levels of biomarkers, such as L-dopa decarboxylase, bombesin-like immunoreactivity, neurospecific enolase, and creatine kinase (9). SCLC-V does not have some markers of neuroendocrine differentiation (L-dopa decarboxylase enzyme activity and bombesin-like immunoreactivity), grows in loose aggregates, and has a more rapid growth rate and higher cloning efficacy than classic SCLC cell lines (17, 18). Extrapulmonary small cell carcinoma is morphologically similar to SCLC and is derived from a variety of sites (19, 20).

IA-1 Gene Expression. Northern blot analysis of IA-1 gene expression was described previously (14). Briefly, 20 μg total RNA were fractionated by 1% agarose/formaldehyde gel electrophoresis and transblotted to Nytran via capillary blotting. The quality and quantity of electrophoresed RNAs were verified either by the intensity of ethidium bromide staining of 18S/28S rRNAs or by rehybridization of the same blot with glyceraldehyde 3-phosphate dehydrogenase cDNA probe (21). Full length IA-1 cDNA (clone IA-1-18) was random primed with hexamers (BRL, Gaithersburg, MD), labeled with [³²P]-dCTP, and 10⁶ cpm/ml-labeled probe was hybridized to the Northern blot at 50°C for 18 h in a solution of 40% formamide, 5 × 10⁷ mw phosphate-buffered saline-1 mw EDTA, pH 7.4, 10 μg/ml sheared salmon sperm DNA, and 6 × Denhardt’s solution (5 prime-3 prime, Inc., Boulder, CO). Blots were hybridized for 16-18 h, followed by four 30-min washes with 1 × 10⁷ mw phosphate-buffered saline-1 mw EDTA, pH 7.4, 0.1% sodium dodecyl sulfate at 55°C. Nytran filters were exposed at ~70°C with intensifying screen for 16 or 96 h and a 3.0 kilobase IA-1 signal was identified.

Chromogranin A Gene Expression. A 250 base pair fragment (1171-1420 base pairs) encoding COOH-terminal of human chromogranin A (22) was isolated from our insulinoma subtraction library (ISL-153) (14). This random-primed labeled human chromogranin A cDNA probe was used for Northern...
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Table 1: Expression of IA-1, chromogranin A, and L-dopa decarboxylase in human lung cancer cell lines

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IA-1&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Cg. A&lt;sup&gt;b&lt;/sup&gt;</th>
<th>DDC&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P/T&lt;sup&gt;d&lt;/sup&gt;</td>
<td>%</td>
<td>P/T&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Small cell carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCLC-C</td>
<td>22/22</td>
<td>100</td>
<td>15/20</td>
</tr>
<tr>
<td>SCLC-V</td>
<td>6/7</td>
<td>86</td>
<td>4/7</td>
</tr>
<tr>
<td>ExPulSC</td>
<td>2/2</td>
<td>100</td>
<td>2/2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>30/31</td>
<td>97</td>
<td>21/29</td>
</tr>
<tr>
<td><strong>Non-small cell carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSCLC&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0/21</td>
<td>0</td>
<td>0/21</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>4/30</td>
<td>13</td>
<td>8/30</td>
</tr>
<tr>
<td>Carcinoid</td>
<td>3/3</td>
<td>100</td>
<td>3/3</td>
</tr>
</tbody>
</table>

<sup>a</sup> IA-1 and chromogranin A (Cg. A) gene expression in various cell lines was detected by Northern analysis as described in "Materials and Methods."

<sup>b</sup> One unit of enzyme activity is defined as 1 nmol l<sup>14</sup>C<sub>2</sub>CO<sub>2</sub>/h. DDC values of >4 units/mg soluble protein were scored positive.

<sup>c</sup> Number positive/number tested.

<sup>d</sup> SCLC-C, classic SCLC; ExPulSC, extrapulmonary small cell carcinoma.

<sup>e</sup> Do not express chromogranin A and/or DDC.

<sup>f</sup> Express chromogranin A and/or DDC.

A panel of 64 human lung cancer cell lines were examined for the expression of IA-1, chromogranin A, and DDC. Table 1 shows that IA-1 mRNA was expressed in 22/22 classic SCLC cell lines, 6/7 variant SCLC cell lines, and 2 of 2 extrapulmonary small cell carcinoma cell lines. The only variant SCLC cell line which had undetectable IA-1 mRNA, NCI-H841, also had undetectable chromogranin A mRNA and DDC. Representative Northern blots are shown in Fig. 1A.

In contrast to SCLC cell lines, 9 of the 30 NSCLC cell lines had at least one of the neuroendocrine markers, chromogranin A mRNA expression in 8 or DDC in 5. Four cell lines had both chromogranin A mRNA and DDC. Four of these 9 NSCLC cell lines (Fig. 1B) had detectable IA-1 mRNA expression (NCI-H358, NCI-H810, NCI-H1155, and NCI-H1385).

The sensitivity of IA-1 as a marker is illustrated in Table 1, which shows that whereas IA-1 mRNA was detected in 97% of SCLC lines, chromogranin A mRNA and DDC enzyme activity were detected in only 72 and 73% of these cell lines, respectively. In general, there was a high concordance between IA-1 and chromogranin A in the various subtypes of SCLC and NSCLC cell lines (Table 2). High concordance also was observed for IA-1 and DDC in all cell lines except SCLC-V. Only 7 SCLC-V cell lines were examined and the concordance between IA-1 and DDC was 29%.

The present report demonstrates that IA-1 is a candidate marker for neuroendocrine differentiation in human lung cancer. IA-1 expression was detectable in 97% of SCLCs, 100% of extrapulmonary small cell carcinoma, and bronchial carcinoids. In the case of NSCLC, IA-1 mRNA was found primarily in cell lines expressing the neuroendocrine markers, chromogranin A and DDC. The absence of IA-1 mRNA expression in non-neuroendocrine NSCLC lines is consistent with the limited expression of IA-1 reported previously in other non-neuroen-

Table 2: Concordant<sup>a</sup> expression of IA-1, chromogranin A, and l-dopa decarboxylase<sup>b</sup>

<table>
<thead>
<tr>
<th>Cell line</th>
<th>IA-1 and Cg. A</th>
<th>IA-1 and DDC</th>
<th>Cg. A and DDC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P/T&lt;sup&gt;c&lt;/sup&gt;</td>
<td>%</td>
<td>P/T&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>Small cell carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCLC-C</td>
<td>15/20</td>
<td>75</td>
<td>19/21</td>
</tr>
<tr>
<td>SCLC-V</td>
<td>5/7</td>
<td>71</td>
<td>2/7</td>
</tr>
<tr>
<td>ExPulSC</td>
<td>2/2</td>
<td>100</td>
<td>2/2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>22/29</td>
<td>79</td>
<td>24/30</td>
</tr>
<tr>
<td><strong>Non-small cell carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSCLC&lt;sup&gt;e&lt;/sup&gt;</td>
<td>21/21</td>
<td>100</td>
<td>20/20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>26/30</td>
<td>87</td>
<td>26/29</td>
</tr>
<tr>
<td>Carcinoid</td>
<td>3/3</td>
<td>100</td>
<td>3/3</td>
</tr>
<tr>
<td><strong>Total lung carcinoma</strong></td>
<td>51/62</td>
<td>82</td>
<td>53/62</td>
</tr>
</tbody>
</table>

<sup>a</sup> Concordance represents the presence or absence of two markers in a particular cell line.

<sup>b</sup> IA-1 and chromogranin A (Cg. A) gene expression were detected by Northern analysis. DDC expression was detected by enzyme activity.

<sup>c</sup> Number positive/number tested.

<sup>d</sup> SCLC-C, classic SCLC; ExPulSC, extrapulmonary small cell carcinoma.

<sup>e</sup> Do not express chromogranin A and/or DDC.

<sup>f</sup> Express chromogranin A and/or DDC.
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References


Fig. 1. Northern analysis of IA-1 gene expression in SCLC and NSCLC. Total RNA (20 μg) isolated from SCLC (A) and NSCLC (B) cell lines were separated on a 1% agarose/formaldehyde gel and hybridized with 32P-labeled IA-1 cDNA probe. A, SCLC (20 μg) isolated from SCLC (A) and NSCLC (B) cell lines were separated on a 1%

endoctrine carcinomas (14). Since many neuroendocrine lung tumors respond to chemotherapy administration (13), it is of considerable clinical interest to determine whether the expression of IA-1 correlates with response to chemotherapy in patients with NSCLC. Antibodies to the IA-1 protein are currently under development and may be useful for this and other purposes. Whether IA-1 serves as a regulatory protein, secretory peptide hormone, or both (14), remains to be determined. Regardless, the sensitivity and specificity of IA-1 expression associated with human lung cancer cell differentiation, as well as the relationship of IA-1 expression to other neuroendocrine markers, warrants further study.
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