purchase and maintain and has limited applications in diagnostic pathology. Its use in diagnostic medicine is largely confined to the bigger hospitals and medical centers, but any pathologist can refer tissue for examination. The quality of the material is critical in influencing the success of the ultrastructural study, and it is highly desirable to obtain tissue that has been handled and fixed carefully. Formalin-fixed specimens can be used, but they are less satisfactory. Any pathologist who frequently handles lung specimens should have an immediately accessible supply of a suitable fixative solution for electron microscopy (2% buffered glutaraldehyde) and should place in the fixative a thin slice of the tissue (<1 mm thick) from any lung tumor that is known to present a problem by light microscopy. The tissue can remain in the fixative solution indefinitely.

The categories in the revised WHO classification that may be appropriate for poorly differentiated and undifferentiated lung carcinomas are undifferentiated large cell carcinoma, small cell undifferentiated carcinoma, and carcinoid tumor. Pathologists frequently resort to calling a tumor with doubtful evidence of differentiation a poorly differentiated carcinoma, but the term has no clinical relevance beyond connoting a non-small cell carcinoma: if neither glandular nor squamous differentiation is clearly present, the tumor should be classified as an undifferentiated large cell carcinoma. Electron microscopy will detect the presence of even the most minor degrees of differentiation, and may therefore serve to reveal that the tumor is a poorly differentiated adenocarcinoma or squamous carcinoma. There is no evidence that minimal differentiation influences the biologic behavior, and it is not uncommon to find a mixture of squamous and glandular characteristics at the ultrastructural level.

A common difficulty encountered in attempting to classify a lung carcinoma that appears undifferentiated by light microscopy is determining whether it is of large cell or small cell type. Pathologists may take refuge in the term “intermediate variant of small cell carcinoma,” but there is no convincing clinical or ultrastructural evidence to prove that subtyping is justified. Morphometric studies have shown that the cell and nuclear dimensions of undifferentiated large cell and small cell carcinomas overlap, but electron microscopy can separate them in most instances. When mixtures of small cell with non-small cell carcinoma occur, the large cell component is usually differentiated indicating the combined variant of small cell carcinoma. Convincing small cell-large cell carcinomas are difficult to document by electron microscopy.

While small cell lung carcinomas and carcinoid tumors are distinct entities, each displays a range of morphology and functional properties, and the two overlap, forming a broad group of neuroendocrine neoplasms. A small number of atypical endocrine tumors with intermediate characteristics occupy the ill-defined mid-zone of this spectrum. Morphologic criteria for their identification are not clearly defined, and the nomenclature used to designate them is confusing. Consequently, these tumors are difficult to classify by light microscopy, and to provide a better understanding of their nature and behavior, further studies by immunostaining and electron microscopy, with clinical correlation, are required.

**REFERENCES**


**Advances in the Biology of Lung Cancer**

**Clinical Significance of Neuroendocrine Differentiation**

*Adi F. Gazdar, M.D.*

On clinical grounds, lung cancers may be divided into two broad subgroups: small cell lung cancer (SCLC), and non-SCLC (NSCLC) cancer. SCLC tumors are relatively sensitive to cytotoxic therapies initially, but later relapse at which time they are resistant to most agents (multidrug resistance). In contrast, most NSCLC tumors demonstrate multidrug resistance at the time of diagnosis. There are also important biologic reasons for this basic subdivision. SCLC is a typical neuroendocrine (NE) tumor. In this report, we will discuss the biologic properties of the various NE tumors of the lung, and discuss the possible effects of this form of differentiation on clinical response.

The primary function of NE cells is the production of specific peptide and amine products. In addition to these specific products, NE cells share many common properties. Included among these general markers are the presence of cytoplasmic dense core cytoplasmic granules, the storage site of the specific products. The granules contain chromogranin A, a matrix protein, and NE cells express L-dopa decarboxylase, an enzyme essential for amine production. The bronchial carcinoid also is a typical NE tumor, with characteristic morphologic features and a more indolent course than SCLC. While bronchial carcinoids are relatively low-grade malignancies, they are resistant to chemotherapy. We, and others, have found that about 12% of NSCLC tumors and cell lines express multiple NE markers. Most of these tumors are adenocarcinomas and large cell carcinomas, while NE differentiation is rare in squamous cell carcinomas. Tumors morphologically similar to SCLC may

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occur in many organs, especially the GI tract, the GU system and in the head and neck. These extrapulmonary small-cell carcinomas (ExPuSC) often have a more indolent course than SCLC, and many respond to cytotoxic therapy. About 50% of the ExPuSC tumors express NE markers, while the remainder probably are other forms of poorly differentiated tumors with only a morphologic similarity to SCLC.

Thus, there are at least 3 morphologically distinct types of pulmonary NE tumors, as well as tumors similar to SCLC arising in extrapulmonary locations. The estimated incidences of these 4 NE tumor types are presented in Table 1. While SCLC is a common tumor, bronchial carcinoids and ExPuSC are considerably rarer. The NSCLC-NE tumors account for only 12% of NSCLC, but, as the latter is one of the most common forms of malignancy in the USA, the incidence of the former also is considerable, with an estimated frequency in excess of that for Hodgkin's disease.

We have established multiple continuous cell lines from all of these tumor types, and have compared and contrasted their properties with those of other, more conventional forms of NSCLC lines. A summary of these findings is presented in Table 2, and they are discussed in the following sections.

While most epithelial tumor cell lines, including NSCLC, demonstrate substrate attachment, most SCLC cultures lack such features and grow as floating cell aggregates.\(^7\) We have observed that carcinoid, NSCLC-NE and ExPuSC cultures usually lack substrate attachment, similar to SCLC and neuroblastomas. Thus, lack of substrate attachment appears to be a characteristic of many forms of NE cells and may be related to the major differences in cell surface glycoproteins previously noted between SCLC and NSCLC.\(^8\)

We have performed in vitro drug sensitivity testing on a large panel of lung cancer cell lines established from previously untreated patients.\(^7\) We used a semi-automated tetrazolium dye (MTT) method to determine accurately the IC\(_\text{50}\) values. Out data indicate that while there is a wide range of values for all tumor types, the median values of 6 cytotoxic drugs for SCLC lines are significantly lower than those of NSCLC or carcinoid. Of particular interest, the values for 4/5 NSCLC-NE lines were similar to those of the SCLC lines, and significantly lower than the values for NSCLC and carcinoids. These findings suggest that expression of NE features in NSCLC tumors may identify a subset that is more responsive to chemotherapy. This hypothesis is currently being tested in a clinical trial being conducted at the National Cancer Institute.\(^9\) Immunohistochemical techniques were used to identify 3 NE markers in 98 NSCLC patients. At least 2 of the markers were present in 20%. Patients received standard therapy followed by chemotherapy at relapse or when appropriate. Response rates were as follows: NSCLC-NE 4/8 (50%) vs 7/34 (21%) for other NSCLC cases. Of all patients who received chemotherapy, 36% had NSCLC-NE, while 88% with progressive disease had other NSCLC. While these differences are not significant, and require more cases before they can be fully evaluated, they suggest a trend for NSCLC-NE tumors to be associated with increased response to chemotherapy.

As mentioned previously, multidrug resistance, occurring either de novo or post-therapy, is a common occurrence in all types of lung cancers. While there are several possible mechanisms by which such multidrug resistance can occur, the best studied form is expression of a membrane B-glycoprotein associated with drug efflux, and which is coded by the MDR1 gene.\(^10\) We have studied expression of the MDR1 gene in normal and malignant lung tissues and in lung cancer cell lines. With the exception of drug-sensitive NSCLC-NE and drug-resistant carcinoid tumors, expression of MDR1 was low in all forms of lung cancer and did not correlate with in vitro sensitivity or with clinical response.\(^11\)

We conclude that mechanisms other than MDR1 expression are responsible for multidrug resistance in lung cancer. Unless verapamil and other calcium channel blockers reverse drug resistance by mechanisms other than affecting drug efflux, our data suggest that they will be ineffective in lung cancer.

In 1982, Whang-Peng examined our lung cancer cell lines and noted a specific chromosomal abnormality associated with SCLC, an interstitial deletion, 3p(14-23).\(^12\) Initially her findings were greeted with skepticism, and the precise incidence of chromosome 3p abnormalities in SCLC was highly controversial. However, using restriction fragment length polymorphism probes, her findings have been confirmed and extended by several laboratories.\(^13\) Recently, Harbour, Kaye and others\(^14\) in our group have found frequent abnormalities of the retinoblastoma (Rb) gene in SCLC tumors and lines. The abnormalities may be either structural, of RNA expression, or both. These findings strongly suggest that loss of genetic material (anti- oncogenes) on chromosomes 3 and 13 (and perhaps on other chromosomes) may play a role in the pathogenesis of SCLC. While similar

<table>
<thead>
<tr>
<th>Tumor type</th>
<th>NE cell Properties</th>
<th>Substrate Attachment</th>
<th>In vitro Sensitivity</th>
<th>MDRI gene Expression</th>
<th>3p Deletion</th>
<th>Rb gene Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCLC</td>
<td>P</td>
<td>A</td>
<td>S</td>
<td>L</td>
<td>P</td>
<td>P</td>
</tr>
<tr>
<td>NSCLC</td>
<td>A</td>
<td>P</td>
<td>R</td>
<td>L</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>NSCLC-NE</td>
<td>P</td>
<td>A</td>
<td>S</td>
<td>H</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Carcinoid</td>
<td>A</td>
<td>P</td>
<td>R</td>
<td>H</td>
<td>P</td>
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<tr>
<td>ExPuSC</td>
<td>P</td>
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<td>S</td>
<td>L</td>
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</tbody>
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P = usually present; A = usually absent; S = relatively sensitive; R = relatively resistant; L = relatively low; H = relatively high.
abnormalities may occur in NSCLC, their incidences are considerably lower. Carcinoid cell lines have similar genetic abnormalities as SCLC, while the NSCLC-NE cell lines appear similar to other NSCLC lines. Of great interest, the ExPuSc lines have a unique phenotype. While they lack deletions of chromosome 3p,\(^9\) they have frequent abnormalities of RB gene (Kaye, submitted).

Table 2 summarizes the properties of the various NE tumors of the lung and of extrapulmonary origin, and compares and contrasts them to non-endocrine NSCLC tumors. Each tumor type has its own unique phenotype. SCLC and carcinoids appear to share similar genetic changes, and thus, may arise through common pathogenetic mechanisms. However, carcinoids are not directly associated with smoking, they are highly chemoresistant, and have a very different clinical course. The NSCLC-NE tumors express the same endocrine properties as the other NE tumors, but their genetic changes appear similar to those of other, non-endocrine NSCLC. Their in vitro chemosensitivity profiles suggest that there may be clinical benefits for identifying this NSCLC subgroup prospectively and treating it vigorously. The ExPuSc tumors frequently express NE markers, and have some, but not all of the genetic changes associated with SCLC. They probably arise from NE cells present in many organs, but by different pathogenetic mechanisms than SCLC. It remains to be determined whether those extrapulmonary tumors that express NE markers differ in their clinical course or response to therapy than tumors that are non-endocrine.

REFERENCES


Bronchial Television Endoscopy

Shigeto Ikeda, M.D., F.C.C.P.*

Since our development of the world’s first flexible bronchofiberscope in 1966, we have been making developments and improvements of bronchofiberscopes for various purposes, such as standard types, biopsy types, image recording types, and small-diameter models. They are currently utilized practically in the clinical applications, and more than 50,000 bronchofiberscopes are now in use throughout the world. These bronchofiberscopes may be considered state-of-the-art both in mechanism and image quality.

We have also been trying to improve the recording capabilities of the bronchofiberscope for taking still photographs and dynamic images, particularly of television images of good quality.

DEVELOPMENT OF BRONCHIAL TV ENDOSCOPE

In February 1987, we developed the first model of a TV bronchoscope with a TV camera built into its tip, of 6.8 mm in diameter, with the cooperation of Asahi Pentax Company. When I took hold of this TV scope for the first time, I felt the same enthusiasm as with the first flexible bronchofiberscope on July 23, 1966. Its outside view is similar to that of the current flexible bronchofiberscope, but the endoscopic images cannot be seen with the eyes. The diameter of the tip is 6.8 mm, that of the flexible part 6.3 mm, and its angle deflections are up 160° and down 100°, and the rigid tip is a little longer than that of the current fiberscope. The angle of its field of view is 100°, and the inside diameter of its biopsy channel is 1.0 mm, which can be used for suction performance but not for biopsy forceps insertion. The TV system used for this scope is an RGB (red, green and blue) field sequential type, with effective pixels of about 32,000.