Synopsis

Twenty-eight cases of primary lung cancer were studied by electron microscopy in detecting ultrastructural differences between cancer cells and non-malignant cells of human lungs.

In a lung cancer tissue, three kinds of cancer cells are observed; dark cells, light cells, and goblet cell-like cells (presently designated as mucous cells). The latter cells are commonly observed in adenocarcinoma and uncornified epidermoid carcinoma of peripheral origin, but rarely in cornified epidermoid carcinoma and undifferentiated carcinoma of central origin.

The cancer cell nucleus is variable in size, shape, and structure. The chromatin clumps of cancer cells are irregularly distributed throughout the nuclei and their size and shape are very variable, unlike those of non-malignant cells. Undifferentiated carcinoma cells (small cell type) are often strikingly characterized by many tiny nucleoli while they reveal a marked condensation of chromatin clumps.

Many abnormal findings are observed suggesting an incomplete maturation or differentiation of cancer cells, i.e., incomplete polarity, long microvilli or striated borders instead of cilia, extraordinarily developed Golgi apparatus or endoplasmic reticulum, etc. Desmosomes and tonofibrils are abundant in cornified epidermoid carcinoma, a few in uncornified epidermoid carcinoma and adenocarcinoma, and none in undifferentiated carcinoma. On ultrastructure, it is impossible to differentiate uncornified epidermoid carcinoma from adenocarcinoma of the lung periphery.

Introduction

Despite a considerable number of reports recently made on electron microscopic studies of tumor cells, the ultrastructure of human lung cancer has been studied by only a few workers, such as Sawada, Edwards, Sasaki, Stoebner, Nagaishi, Bensch, and Watson.

An important thing for electron microscopic studies of cancer cells is a technique of specimen preparation. Exploration of a fine nuclear structure has been highly facilitated by the introduction of double fixation (prefixation with Formalin or glutaraldehyde, and postfixation with osmium tetroxide), embedding in Epon, and staining of nucleic acids with uranyl ions or lead. As Fawcett stated, a general appearance of the chromatin after this kind of specimen preparation corresponds quite closely to the familiar picture seen with the light microscope in tissues stained with basic dyes.

Received for publication: 283
These modern techniques are greatly contributing to the exploration of nuclear fine structure and cytoplasmic membraneous structure. The present study was carried out to detect ultrastructural differences between cancer cells and non-malignant cells of human lungs.

**Materials and Methods**

Twenty-eight cases of primary lung cancer were studied histologically and electron microscopically (Table 1). Immediately after surgical operation, small pieces (approximately 2 mm thick) of tumorous and non-tumorous lung tissues were fixed in cold 6% glutaraldehyde solution, buffered with phosphate to pH 7.2, for 30 mins. and then in cold 2% osmium tetroxide solution in phosphate buffer (pH 7.2) for additional 1 hour. All tissues for electron microscopic study were embedded in epoxy resin (Epon 812) by the method of Luft. Sections were cut with glass knives on the Leitz microtome or LKB microtome, stained with uranyl acetate or lead, and examined with a JEM-6C electron microscope (Nippon Electron Optics).

At the same time, other specimens were fixed in 10% Formalin solution and served for histological examination. Hematoxylin-Eosin staining, Alcian Green staining, and PAS staining were used for histological sections.

**Results**

**Dark Cell and Light Cell**

Electron microscopically, cancer cells look dark (dark cell) or light (light cell or clear cell) depending on the amount of free Palade granules in the cytoplasm. In cases of lung cancer, it is notable that cancer cells of another goblet cell-type intermingle with dark cells and light cells in the same cancer tissue. These goblet-like tumor cells, presently designated as mucous cells, show a high development of rough-surfaced endoplasmic reticulum, which often forms rather enlarged cisternae, so that they are reasonably considered to have a secretory function just like goblet cells (Photos 1 and 2). Distinct desmosomes are visible on the boundaries between these cells and dark or light cells (Photo 2). Ratios among these three kinds of cancer cells are variable depending on histological types. Mucous cells are abundant in tissues of adenocarcinoma, but very few in undifferentiated carcinoma (small cell type or oat cell type) or in cornified epidermoid carcinoma of central origin. Undifferentiated carcinoma (small cell type or oat cell type) is predominantly featured by dark cells (Photos 3 and 4).

**Nuclei and Nucleoli**

Electron microscopic findings of human lung cancer cells are very variable, depending on cellular maturity. Generally, the nuclei of cancer cells show a variety of shapes, sizes, and structures.

In lung cancer cells, chromatin clumps are irregularly distributed throughout the nucleus, and the size and shape of chromatin clumps are very variable. Nuclei of dark cells and mucous cells of lung cancer look denser than those of light cells, and the former chromatin pattern is more irregular and coarser than light cells (Photos 1 and 2). In undifferentiated carcinoma cells (small cell type or oat cell type), chromatin clumps are more condensed, becoming more irregular and larger than those of other differen-
<table>
<thead>
<tr>
<th>Case No.</th>
<th>Name</th>
<th>Age (yrs.)</th>
<th>Sex</th>
<th>Max. diam. (cm)</th>
<th>Site of lesion (involved bronchial obstruction)</th>
<th>Histological findings</th>
<th>Alcian-Green staining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SS</td>
<td>68</td>
<td>M</td>
<td>1.5</td>
<td>r—S₁ (Secondary)</td>
<td>Cornified epidermoid carcinoma</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>TH</td>
<td>70</td>
<td>M</td>
<td>5.5</td>
<td>r—S₁ (&quot; )</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>3</td>
<td>AH</td>
<td>47</td>
<td>M</td>
<td>5.0</td>
<td>r—S₁S₅ (Tertiary)</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>4</td>
<td>MH</td>
<td>52</td>
<td>M</td>
<td>4.5</td>
<td>S₁+₂ (&quot; )</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>5</td>
<td>YK</td>
<td>62</td>
<td>M</td>
<td>4.0</td>
<td>r—Lower (Main)</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>6</td>
<td>MU</td>
<td>65</td>
<td>F</td>
<td>8.0</td>
<td>r—S₁ (Secondary)</td>
<td>Corrinized epid. carcinoma &amp; adenocarcinoma</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>SI</td>
<td>53</td>
<td>F</td>
<td>3.5</td>
<td>S₁+₂ (Quaternary)</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>8</td>
<td>UY</td>
<td>68</td>
<td>M</td>
<td>3.0</td>
<td>1—S₃ (&quot; )</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>9</td>
<td>NK</td>
<td>59</td>
<td>M</td>
<td>4.0</td>
<td>r—S₅ (Tertiary)</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>10</td>
<td>UN</td>
<td>67</td>
<td>M</td>
<td>3.0</td>
<td>r—S₅ (&quot; )</td>
<td>Uncornified epidermoid carcinoma</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>KF</td>
<td>62</td>
<td>M</td>
<td>5.5</td>
<td>r—S₁S₃ (Quaternary)</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>12</td>
<td>EI</td>
<td>69</td>
<td>M</td>
<td>4.5</td>
<td>r—S₂b (5th )</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>13</td>
<td>CS</td>
<td>65</td>
<td>M</td>
<td>5.5</td>
<td>1—S₁ (Quaternary)</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>14</td>
<td>SK</td>
<td>57</td>
<td>M</td>
<td>4.5</td>
<td>1—S₁ (Secondary)</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>15</td>
<td>SO</td>
<td>70</td>
<td>M</td>
<td>2.3</td>
<td>r—S₂b (5th )</td>
<td>Uncornified epidermoid carcinoma &amp; adenocarcinoma</td>
<td>+</td>
</tr>
<tr>
<td>16</td>
<td>TO</td>
<td>68</td>
<td>M</td>
<td>1.5</td>
<td>1—S₉a (Quaternary)</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>17</td>
<td>KT</td>
<td>59</td>
<td>M</td>
<td>3.0</td>
<td>r—S₉ (Secondary)</td>
<td>Uncornified epid. carci. &amp; undifferentiated carci.</td>
<td>—</td>
</tr>
<tr>
<td>18</td>
<td>AT</td>
<td>54</td>
<td>M</td>
<td>2.0</td>
<td>S₁+₂ (9th )</td>
<td>Uncornified epid. carci., adenocarcinoma &amp; undifferentiated carcinoma</td>
<td>+</td>
</tr>
<tr>
<td>19</td>
<td>KF</td>
<td>58</td>
<td>M</td>
<td>3.0</td>
<td>1—S₉a (6th )</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>20</td>
<td>TY</td>
<td>62</td>
<td>F</td>
<td>3.2</td>
<td>r—S₁₉ (Quaternary)</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>21</td>
<td>SF</td>
<td>50</td>
<td>F</td>
<td>5.0</td>
<td>1—S₅ (&quot; )</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>22</td>
<td>TT</td>
<td>55</td>
<td>M</td>
<td>4.0</td>
<td>1—S₉a (Quaternary)</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>23</td>
<td>SN</td>
<td>47</td>
<td>F</td>
<td>3.8</td>
<td>S₁+₂b (5th )</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>24</td>
<td>Ki</td>
<td>47</td>
<td>M</td>
<td>5.5</td>
<td>S₁+₂b (Tertiary)</td>
<td>Cylindroma</td>
<td>+</td>
</tr>
<tr>
<td>25</td>
<td>SK</td>
<td>50</td>
<td>M</td>
<td>4.5</td>
<td>r—S₁ (Secondary)</td>
<td>Undifferentiated carcinoma (small cell type)</td>
<td>—</td>
</tr>
<tr>
<td>26</td>
<td>KN</td>
<td>54</td>
<td>F</td>
<td>9.5</td>
<td>1—S₁S₉ (Tertiary)</td>
<td>&quot;</td>
<td>&quot;</td>
</tr>
<tr>
<td>27</td>
<td>RN</td>
<td>54</td>
<td>M</td>
<td>2.5</td>
<td>r—S₉b (6th )</td>
<td>Undiff. carci. (small cell type) &amp; epidermoid carcinoma</td>
<td>—</td>
</tr>
<tr>
<td>28</td>
<td>FF</td>
<td>53</td>
<td>M</td>
<td>4.5</td>
<td>r—Upper (Main)</td>
<td>Undiff. carci. (large cell type) &amp; epidermoid carcinoma</td>
<td>—</td>
</tr>
</tbody>
</table>
tiated carcinoma cells; they are distributed very unevenly throughout the nucleus and along the nuclear membrane (Photos 3 and 4).

On the contrary, chromatin patterns of non-malignant cells are rather regular. For example, in normal alveolar epithelial cells the chromatin is regularly distributed along the nuclear membrane, a chromatin clump is visible in close contact with a nucleolus (nucleolus-associated chromatin), and a few intranucleolar chromatin clumps are also present.

Nucleoli of lung cancer cells generally consist of nucleolonema and pars amorpha. However, in some cases the nucleolonema without pars amorpha is observed. Irregularity in shape, enlargement, and increased number of nucleoli are common findings of lung cancer cells. It is notable that undifferentiated carcinoma cells (small cell type or oat cell type) occasionally have numerous and very small nucleoli (less than 1.2 μ in diameter), and their nucleolonema remains undeveloped (Photo 4).

Lobulations and deep invaginations are also common features of the nuclei of lung cancer cells, and the nuclear pores are very irregular in their distribution.

In some cases of lung cancer, two types of nuclear inclusions are observed: a fibrillar structure (Photos 5 and 6) and a condensation of small particles.

**General Cell Appearance**

It is generally accepted that cancer cells usually show a larger nuclear-cytoplasmic ratio than normal epithelial cells. A nuclear-cytoplasmic ratio is large in undifferentiated carcinoma cells, but rather small in adenocarcinoma and epidermoid carcinoma cells. Striking characteristics of differentiated carcinoma cells are cellular enlargement and very irregular cell borders (Photo 1).

The loss of polarity is another common finding of lung cancer cells. Occasionally, polarity and gland-like cellular arrangement are visible in adenocarcinoma cells (Case Nos. 19, 21, 22, and 23 in Table I), having long microvilli on the surface cell membrane, distinct terminal bars, and many osmiophilic dense bodies above the nucleus just like ciliated epithelial cells (Photos 7 and 8). These cancer cells with polarity are observed sporadically among irregularly arranged adenocarcinoma cells.

So far, distinct microvilli were observed in 11 out of 28 cases of lung cancer, especially abundant in adenocarcinoma cells. Some microvilli of cancer cells (Case Nos. 12 and 21) show a filamentous structure very similar to that of cilia and it is difficult to differentiate them at low magnification. At high magnification, such microvilli reveal many basal corpuscles, but no filamentous rootlets (Photos 9 and 10).

Mitochondria of cancer cells are generally distributed throughout the cell, showing no preferred location. The size and shape of mitochondria are very variable, and their filamentous matrix is sometimes very dense or empty. Mitochondria seem to be numerous in adenocarcinoma cells, especially in mucous cells, and a few in epidermoid carcinoma cells or in undifferentiated carcinoma cells.

Although Golgi apparatus is said to be a common cytoplasmic organelle in almost all cancer cells, cornified epidermoid carcinoma cells are often devoid of Golgi apparatus, because cytoplasmic organelles of cornified epidermoid carcinoma cells tend to disappear being accompanied with a keratinization of abundant tonofibrils.

It has been disclosed that carcinoma cells, especially undifferentiated carcinoma cells, have a few organelles, as is the case with primitive cells described by Bernhard. How-
ever, an extraordinarily developed Golgi apparatus is observed, although not frequently, in a few cancer cells among a crowd of undifferentiated carcinoma cells (Photos 11 and 12). Also, in adenocarcinoma cells, an extraordinarily developed rough-surfaced endoplasmic reticulum is occasionally observed (Photos 13 and 14).

In a study on bronchial carcinoid tumor, Bensch\(^1\) reported a peculiar apposition of two adjacent cisternae of the rough-surfaced endoplasmic reticulum along the inner opposing membranes of these cisternae. In the present study, such peculiar apposition consisting of three or four parallel membranes was also observed in 8 out of 28 cases of lung cancer.

Generally, secretory granules of cancer cells are more often found in the cytoplasm of mucous cells than in that of light cells or dark cells. However, regardless of cellular types, lipid droplets and cholesterol crystals are frequently observed in various kinds of cancer cell. Serotonin granules,\(^1\) which are common in the cytoplasm of carcinoid tumor, were also observed in cells of undifferentiated carcinoma (Case No. 25 in Table I; Photos 15 and 16). Osmiophilic lamellar bodies, which are the specific inclusion bodies in alveolar wall cells,\(^7,8\) were observed in cancer cells of Case Nos. 8 and 21 out of 28 cases shown in Table I.

It is assumed that the glycogen content is less in cancer cells than in non-malignant cells, because of an active glycolysis in the former. In the present study, abundant glycogen granules were observed in 4 out of 28 cases of lung cancer. Especially in Case Nos. 7, 17, and 19 in Table I, glycogen was abundantly observed in the vicinity of keratinized tonofibrils (Photos 17 and 18).

It is well known that the presence of desmosomes is common to epithelial cells. Although the degree of development of desmosomes in lung cancer is variable depending on histological types, the desmosome develops well in the cell of cornified epidermoid carcinoma. Closely related to the development of desmosomes, tonofibrils in cornified epidermoid carcinoma cells develop well. They show a thick, band-like formation suggesting keratinization.

In adenocarcinoma cells the development of tonofibrils and desmosomes is scanty. In undifferentiated carcinoma cells (small cell type), tonofibrils and desmosomes are very scarce, showing a marked contrast with abundant tonofibrils in basal cells of the normal bronchial epithelium or cells of the normal bronchial mucous gland.

**DISCUSSION**

As already described, the progress of modern techniques of specimen preparation in the electron microscopic field enabled us to study a nuclear fine structure as well as a cytoplasmic membraneous structure of cancer cells.\(^2,5,12\) It seems reasonable and significant to make a comparative study of the ultrastructure of malignant cells with that of non-malignant cells by the modern electron microscopic techniques.

The most striking feature of the cancer cell nucleus is a great variability of its size, shape, and structure. Generally, chromatin clumps of cancer cells are irregularly distributed throughout the nuclei which are different in their size and shape.

In the lung cancer tissue, three kinds of cancer cells are observed; dark cells, light cells, and mucous cells. Mucous cells are commonly observed in adenocarcinoma and uncornified epidermoid carcinoma of peripheral origin, but rarely in cornified epidermoid
carcinoma and undifferentiated carcinoma of central origin. They are richer in nuclear
density and chromatin condensation than light cells, suggesting high secretory activity.
As they are considered to be rather mature type, it is reasonable that they are seldom
seen in a rapidly growing undifferentiated carcinoma (small cell type).

Generally, undifferentiated carcinoma cells (small cell type) are scanty in their
organelles and devoid of desmosomes and tonofibrils unlike epidermoid carcinoma and
adenocarcinoma cells. In undifferentiated carcinoma cells a nuclear-cytoplasmic ratio
is markedly increased, and abundant chromatin clumps are unevenly distributed
throughout the nuclei. Their nucleoli are rather small in size but large in number,
and their nuclear pores are irregularly distributed. From these findings it is assumed
that an extensive energy consumption is required only for an intensive mitotic activity
rather than a cellular maturity.

The other abnormal findings in cancer cells are an extraordinary development of the
Golgi apparatus in some undifferentiated carcinoma cells (Photos 11 and 12) and an
unexpected development of the endoplasmic reticulum in some adenocarcinoma cells
(Photos 13 and 14), suggesting disorders of differentiation.

Microvilli are commonly observed in many lung cancer cells, whereas cilia are never
observed even in cancer cells of central origin.

Although some adenocarcinoma cells show polarity just like ciliated epithelial cells,
their cellular structures are incomplete, i.e., they show long microvilli or a striated
border instead of cilia, phospholipid granules of irregular shape in the upper part of
nucleus, and irregularly shaped mitochondria in contrast to normal ciliated epithelial
cells (Photos 7-10). Such structural findings seem to evidence an incomplete maturation
of cancer cells.

The other striking features of lung cancer cells are represented by lobulation and
deep invagination of nuclei, larger nuclear-cytoplasmic ratio, cellular enlargement, and
irregularity of the cell border which are familiar findings in a cytologic diagnosis of
cancer cells.

In a case of undifferentiated carcinoma (small cell type), peculiar intranuclear fibrillar
formations were observed. Although this structure is very similar to that seen in the
cytoplasm of leukemia cells, it is still obscure whether this is pathognomonic to lung
cancer.

Mitochondria of cancer cells are variable in size, shape, and structure. They are
distributed unevenly, and their filamentous matrices are very irregular. It is impossible
to make a quantitative estimation of mitochondria in an electron microscopic study.

Although there are many kinds of osmiophilic dense bodies in lung cancer cells,most interesting bodies are osmiophilic lamellar bodies and serotonin granules from the
viewpoint of carcinogenesis, which will be discussed in the following paper.

(Received December 27, 1966)
REFERENCES


EXPLANATION OF PLATES XLII-L

Photo 1. Three kinds of cancer cells are observed in a lung cancer tissue (Case No. 14 in Table I); dark cell (Dc), light cell (Lc), and goblet cell-like cell (Mucous cell: Mc). Mg: Mucous gland.

Photo 2. High power view of Photo 1. Desmosomes (D) are visible between light cell (Lc) and mucous cell (Mc). Nuclei of mucous cells of lung cancer look denser than those of light cells, and the chromatin pattern of the former is more irregular and coarser than those of the light cells.

Photo 3. Undifferentiated carcinoma of oat cell type (Case No. 25 in Table I). Chromatin clumps are more condensed, becoming more irregular and larger than those of other cancer cells in Photos 1 and 2.

Photo 4. Undifferentiated carcinoma of small cell type (Case No. 27). Chromatin pattern is quite the same as Photo 3. Almost all cells are dark cells (Dc), and their organelles are few. No desmosomes and no tonofilaments are visible. Note many tiny nuclei (Nl). Mt: Mitosis.

Photo 5. Undifferentiated carcinoma of oat cell type (Case No. 25). Unusual nuclear inclusions showing fibrillar structure (Fi) are visible.

Photo 6. High power view of Photo 5.

Photo 7. Normal bronchial epithelial cells showing polarity. Mitochondria (M) are small in size and regular in shape, and concentrated in upper part of cells. Many phospholipid granules (Li) are visible above the nuclei. C, cilia; Gc, goblet cell; Bc, basal cell; G, Golgi apparatus.

Photo 8. Incomplete polarity of cancer cells (Case No. 19), showing long microvilli (Vi) instead of cilia, distinct terminal bars (T), and many phospholipid granules (Li). However, size of phospholipid granule is very small in contrast with marked cellular hypertrophy. The size and shape of mitochondria are very variable, and their filamentous matrices are irregular.

Photo 9. Gland-like cellular arrangement of adenocarcinoma cells (Case No. 21). They have long microvilli (Vi) on the surface cell membrane, terminal bars (T), osmiophilic dense bodies (Li), and Golgi apparatus (G).
Photo 10. High power view of Photo 9. These microvilli show a filamentous structure very similar to that of cilia and reveal many basal corpuscles. However, no filamentous rootlets are observed. It is reasonable to call them striated borders.9)

Photo 11. Undifferentiated carcinoma of small cell type (Case 27). As shown in Photo 4 (same case), almost all cancer cells have few organelles. However, like this photograph, an extraordinary development of Golgi apparatus (G) in some cancer cells is observed, suggesting a disorder of differentiation.

Photo 12. High power view of Photo 11.

Photo 13. An extraordinary development of endoplasmic reticulum (Er) is occasionally observed in some adenocarcinoma cells (Case 18).


Photo 15. Granules very similar to serotonin granules are observed in cells of an undifferentiated carcinoma of oat cell type (Case No. 25).

Photo 16. High power view of Photo 15.

Photo 17. Abundant glycogen granules (Gl) are observed in some cells of cornified epidermoid carcinoma (Case No. 7).

Photo 18. High power view of Photo 17. Glycogen granules are observed in the vicinity of keratinized tonofibrils. Gl, glycogen granules; Tf, tonofibrils, D, desmosomes.