ABSTRACT
A feeder layer culture system suited to grow carcinoma cells derived from solid human lung tumors was developed. This report deals with culturing of the four main histological types of lung carcinomas observed in 37 patients: squamous cell, adenocarcinomas, small cell, and large cell carcinomas. The cultures were initiated from 24 fresh human surgical specimens and from 14 human lung tumors grown as xenografts in nude mice.

Three different patterns of behavior in culture were found to be characteristic for squamous cell, adenocarcinomas, and small cell carcinomas, respectively. The culture pattern presented by the primary cultures did not appreciably change after passaging in vitro for periods of up to 2 years, even after infinite cell lines were established. Cultures of large cell carcinoma showed one or more of these patterns. From these patterns cells could be cloned and subsequently cultured as separate stable lines. The system described facilitates the identification of specific types of human lung carcinomas almost immediately (within 1 h) after plating (Phase I) as well as during culture.

INTRODUCTION
Human lung carcinoma (1) cell cultures were initiated in order to develop established carcinoma cell lines. Because lung cancer cells especially non-small cell tumors are difficult to grow (2), a modified feeder layer culture method using a complete sheet of feeder cells was developed.

This culture system proved to be selective for the growth of malignant cells; all nonmalignant epithelial and connective tissue cells die within two days. Moreover, it allows serial culturing of carcinoma cells and therefore the establishment of continuously growing tumor lines and the cloning of these lines. A characteristic behavior and growth pattern was observed that retrospectively could be correlated with the histological classification of the tumor of origin.

MATERIALS AND METHODS
Tumor Material. Fresh tumor material was obtained from 37 patients with histologically proven lung carcinoma. In 24 cases surgical specimens were directly cultured in vitro and developed into cell lines named, RLTH/pt.1 In 13 cases tumor material was first transplanted into nude mice for one or more passages (n) and subsequently cultured in vitro (RLTH/n). In one case both procedures were followed. Cells from some established cell lines were (re)transplanted into nude mice and after a take reintroduced into culture as well as into nude mice. This offers the possibility to prove their malignancy in vivo and to use tumor cells of the same passage for in vitro and in vivo studies of sensitivity for chemotherapeutic drugs and radiation. To evaluate the culture system, other human and rat tumors as well as nonmalignant cell lines and freshly derived bone marrow cells were also used.

Culture System. Minced tumor material was grown on top of a complete sheet of feeder cells in Dulbecco's modified Eagle's medium containing 10% newborn calf serum and a maintenance dose of 100 IU/ml penicillin and 0.1 mg/ml streptomycin. The feeder layer system was prepared by inoculating 8 x 10^4 cells of a nonmalignant murine C3H10T1/2 cell line (3) in 25-cm² culture flasks. After about 10 days of culture these cells formed a complete sheet and entirely covered the surface of the culture flasks. At that time the culture was irradiated with a dose of 50 Gy γ-rays. Immediately afterwards or after a few days the irradiated cultures were used for growing the tumor cells. As feeder layer cells we tried also 3T3 cells, but the 3T3 sheet was less strongly attached to the plastic surface and detached within about a week. C3H10T1/2 cells on the other hand can be used up to many weeks, dependent on the growth activity of the tumor cells. Cells of three nonmalignant clones of a mouse spleen cell strain (4) as well as two nonmalignant cell strains derived from rat embryos formed good sheets, but these were less firmly attached to the surface than the C3H10T1/2 cell sheets, moreover, they did not support the growth of the tumor cells as well as C3H10T1/2 cells did.

Our culture system differs from the feeder layer technique introduced by Puck et al. (5) for cell cloning in that in the latter system feeder cells and tumor cells are inoculated at the same time. Furthermore, feeder cells are plated in such a proportion that one-third of the surface of the flask can be occupied by feeder cells. This offers nonmalignant cells present in the tumor suspension the possibility to anchor directly onto the surface of the flasks and give rise to colonies. Both systems make use of the metabolizing capacities of the feeder cells to condition the medium and to sustain growth of nonirradiated tumor cells at low cell concentrations. We found it essential for selective growth of tumor cells, including aggregated carcinoma cells, that the whole surface of the flask be covered with well attached feeder cells.

The freshly received tumor material was divided into two parts which are handled in the same way but separately. For each sample, were used for transplantation into nude mice and for histology; the remaining tissue was minced with a pair of scissors and used for culturing. No enzyme treatment was used. Minced tumor material of both parts was inoculated into two (one odd and one even number) feeder layer cultures each, resulting in four primary cultures per tumor.

The remaining tumor mince was kept in reserve as monolayer culture without feeder layer. Part of this material had to be used in a few cases when not enough viable tumor cells appeared in the primary culture. Once or twice a week the medium was partly or completely renewed depending on the growth activity of the carcinoma cells. Cultures were passaged onto a fresh feeder layer when the feeder lay deteriorated or after the carcinoma cells formed relatively large compact sheets. Passaging may become necessary after 1–3 days with primary or secondary cultures and up to 6 weeks in the phase of slow growth preceding the development of an immortal cell line. For passaging, the feeder layer with the tumor cells was mechanically detached with a silicone policeman (Fig. 1, T) and minced by pipetting. The cultures were inspected and their culture behavior was described shortly after seeding and at least twice weekly. At various intervals during culturing photomicrographs were taken by using an inverted microscope without phase contrast.

Some parallel cultures, especially from small cell tumors, were set up without feeder layer in HITES medium as described by Carney et al. (6). Data derived from such cultures were not used for this report.

Cloning. Carcinoma cell lines were cloned by picking up a circumscribed group of tumor cells plus underlying feeder layer with a bent Pasteur pipet. The cell clumps were broken up by pipetting, and the resulting suspension was plated onto a new feeder layer. Cell clusters and a few viable tumor cells, probably cells that were dividing at the time of transfer, settled and grew. These dividing cells were marked on the bottom of the flask and after they gave rise to a clone were used to start the cell strain.

Malignancy Test in Nude Mice. Cell lines considered to be immortal were tested for malignancy by injecting several samples of about 10⁶ cells s.c. into nude mice. All cell lines tested so far produced progres-
Fig. 1. Histological appearance and pattern of in vitro cultures of human SqCC of the lung. T, cluster of SQCC cells (RLTH 17/4) covered with old feeder layer cells derived from the phase III of culturing after loosening from the bottom by scraping with a policeman. The cluster is already attached to the new feeder layer. Tumor cells move out to cover the feeder layer. × 100. I, histological appearance of a well differentiated SqCC (RLTH 17/pt) showing epithelial pearls and keratinization. Hematoxylin-phloxine-saffron × 120. II, spherule of well differentiated SqCC (RLTH 17/4) attached to the feeder layer 3 hours after plating. Halo (arrow) indicates the smooth surface consisting of a compact layer of cells. × 150. III, “fried egg” shape of well differentiated SqCC (RLTH 17/4) 1 day after plating. The spherule has flattened and cells have moved out and start covering the feeder layer. × 150. IV, 3–7 days after plating a compact epithelial monolayer is formed with a sharply defined edge. Growth is concentric. Dividing cells can be found anywhere within the sheet. Those cells which do not fit into the monolayer form squamae (arrows) of flattened dead cells (RLTH 17/4). × 72. V, higher magnification of the central area of the compact epithelial monolayer of the well differentiated SqCC (RLTH 17/4). Ultrastructural examination (not shown) revealed many desmosomes between these cells. × 550.

Differentially growing tumors after a relative long latency (1–4 months). Ninety % of the samples inoculated were positive. Histology. Histological preparations were made from tumor material taken during surgery, from parts of the tumors used to start the cultures, and from pieces of the tumors arising in nude mice after inoculation of cells of the established tumor lines. The material was classified according to the revised WHO classification of lung tumors (1981) (1) using its definitions and (light microscopic) diagnostic criteria. To judge whether the tumor sample used for transplantation and culture was representative for the complete material as seen by the clinical pathologist, diagnosis and description of the clinical pathologist and that of the research pathologist (C. Z.) were compared in each case.

Based on the diagnosis of the clinical pathologist the material could be classified into one of the four most common categories, i.e., SqCC, AdC, SCLC or LCC. Subclassification was applied whenever possible. Bronchioloalveolar carcinoma, defined as an adenocarcinoma in which cylindrical tumor cells grow upon the walls of preexisting alveoli, cannot be diagnosed in s.c. transplants in nude mice; therefore, subclassification of such tumor transplants was limited to acinar or papillary carcinomas.
Differential Behavior of Human Bronchial Carcinoma Cells

When introduced into culture, the carcinoma cell clusters attached, mostly within a few hours, to the feeder layer. They remained healthy, began to spread over the feeder layer (Fig. 2, Phase II) and started growing. In the ensuing days a monolayer or double layer of carcinoma cells was formed over the feeder layer (Fig. 2, Phase III). The nonmalignant cells also settled onto the feeder layer; however, these cells shrunk and deteriorated within a few days. The same holds for single tumor cells that do not cluster and are doomed to die. At places not covered by feeder layer cells, connective tissue cells can attach to the surface of the flasks and grow. After transfer of such cultures onto complete feeder layers, these nonmalignant cells will die because they cannot grow as a double layer on top of a feeder layer. The only normal cells that were found to grow well on feeder layer were freshly derived bone marrow cells.

Passage of Culture

The mechanically detached and minced feeder layer with tumor cells was transferred onto new feeder layers. The cell aggregates with or without feeder cells attached within three hours. In general, carcinoma cells grow well during the early passages, but growth slows down afterwards. After 6–8 passages over a period of 2–3 months in the case of mouse and over a period of 3–6 months for rat tumors and after a resting phase of 6–12 months in case of human tumor cells, growth accelerates and an infinite tumor cell line has developed. This change occurs around the same time in the various parallel cultures of the same tumor. While the immortalization of nonmalignant mouse and rat cells is accompanied by phenotypic changes towards uniformity of the cells, the cellular aspect and behavior of our tumor cells hardly changed. In fact the immortalized cells resemble those in the active growth phase of the early passages. These immortalized tumor cells are also able to grow without feeder layer. The squamous cell sheets are often difficult to transfer onto flasks without feeder layer, because they do not attach. To prevent selection, part of these cultures were kept growing onto feeder layers to maintain differentiated tumor cell cultures.

Differential Behavior of Bronchial Carcinoma Cells

So far, this culture method has been used with fresh surgical specimens from 24 patients with lung carcinomas and from 14 human lung carcinomas grown in nude mice. Based on the histological diagnosis of the clinical pathologist, primary cultures were derived from 19 SqCC, 6 AdC, 7 SCLC, and 5 LCC (Tables 1–3; Figs. 1 and 3–5). Many of these tumors were also passaged in nude mice, which provided the possibility to start cultures derived from the same tumors more than once. All cultures derived from fresh histologically tumor positive material were successful. During the three phases of culturing (I, II, and III) (see below) three main patterns (types A, B, and C) of behavior in culture were observed (Figs. 1–5). These patterns were retrospectively compared with the original histological diagnosis (Tables 1–3). No essential differences with respect to growth and behavior pattern were found between the four primary cultures or subsequent passages obtained from the same tumor.

Phase I: Formation of Cell Aggregates. Just before and after plating, aggregates of tumor cells which are different for types A, B, and C are formed directly or within 3 hours. A compact spherule with a clear halo is formed in case of type A behavior. In type B, irregular, loosely packed cell structures are seen, while type C is characterized by the formation of spherules
DIFFERENTIAL BEHAVIOR OF HUMAN BRONCHIAL CARCINOMA CELLS

Table 2 Behavior pattern in culture of human lung carcinomas after passage in nude mice

<table>
<thead>
<tr>
<th>RLTH no.</th>
<th>Histological diagnosis</th>
<th>Passage no. in nude mice</th>
<th>Histological diagnosis of tumor transplant in nude mouse</th>
<th>Pattern in culture (type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>SqCC m</td>
<td>n 4</td>
<td>SqCC WD</td>
<td>A</td>
</tr>
<tr>
<td>32</td>
<td>SqCC p</td>
<td>n 6</td>
<td>SqCC WD</td>
<td>A</td>
</tr>
<tr>
<td>44</td>
<td>SqCC p/m</td>
<td>n 3</td>
<td>SqCC WD</td>
<td>A</td>
</tr>
<tr>
<td>45</td>
<td>SqCC w</td>
<td>n 1</td>
<td>SqCC WD</td>
<td>A</td>
</tr>
<tr>
<td>13</td>
<td>AdC m</td>
<td>n 2</td>
<td>SqCC WD</td>
<td>A</td>
</tr>
<tr>
<td>40</td>
<td>AdC p</td>
<td>n 1</td>
<td>Undifferentiated carcinoma</td>
<td>B, C</td>
</tr>
<tr>
<td>39</td>
<td>SCLC</td>
<td>n 3</td>
<td>SCLC</td>
<td>C</td>
</tr>
<tr>
<td>49</td>
<td>SCLC</td>
<td>n 1</td>
<td>SCLC</td>
<td>A, C</td>
</tr>
<tr>
<td>83</td>
<td>SCLC</td>
<td>n 6</td>
<td>SCLC</td>
<td>A, C</td>
</tr>
<tr>
<td>84</td>
<td>SCLC</td>
<td>n 7</td>
<td>SCLC</td>
<td>A, C</td>
</tr>
<tr>
<td>16</td>
<td>LCC</td>
<td>n 6</td>
<td>SCLC</td>
<td>A, C</td>
</tr>
<tr>
<td>26</td>
<td>LCC</td>
<td>n 9</td>
<td>SCLC</td>
<td>A, C</td>
</tr>
<tr>
<td>35</td>
<td>LCC</td>
<td>n 10</td>
<td>SCLC</td>
<td>A, C</td>
</tr>
</tbody>
</table>

* WD, well differentiated; PD, poorly differentiated; MD, moderately differentiated; NA, not available.
* After fourth passage in nude mice cultured in vitro for 3.8 years followed by two passages in nude mice.
* Focal cribriform aspect.
* After first passage in nude mice, cultured and cloned in vitro for 10 months followed by five nude passages of cloned cells.
* Large cells in culture.
* Difficult to classify; split diagnosis AdC or SCLC intermediate cell type.

Table 3 Behavior patterns in vitro of human lung carcinomas cultured directly (pt) or after passage in nude mice (n)

<table>
<thead>
<tr>
<th>Histological Diagnosis</th>
<th>Origin culture (no.)</th>
<th>Pattern type in culture A/B/C</th>
<th>Pattern type in culture A/B/C</th>
<th>Pattern type in culture A/B/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>SqCC</td>
<td>pt (15)</td>
<td>14</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>n (4)</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>AdC</td>
<td>pt (4)</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>n (2)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SCLC</td>
<td>pt (3)</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>n (4)</td>
<td>3</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>LCC*</td>
<td>pt (1)</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>n (4)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* LCC (RLTH65) was cultured directly and after passage in nude mouse. Both showed pattern A in culture.

which instead of a halo show separated “cobblestone” shaped cells on their surface.

Phase II: Attachment of Aggregates onto the Feeder Layer and Remodelling. Directly after plating, the cells and cell aggregates settled on top of the feeder layer and formed different structures of types A, B, and C. Within 1-4 days, type A aggregates showed transition into a “fried egg” shape. Type B aggregates attached to the feeder layer; they spread and formed double layers with focal extensions (“streamers”) consisting of viable cells. Finally, type C cells were rearranged as a pile of loosely packed cells. Only cells in contact with the feeder layer attached.

Phase III: Growth and Development of Monolayers or Double Layers of Carcinoma Cells. Also in this phase III patterns are observed. In type A cultures, the cells showed concentric growth in monolayers. Some of the dividing cells ended up as double layer, but these cells died and remained as loosely attached or free floating squamae. In type B cultures, the cells formed incomplete double layers of viable cells with streamers on top. The streamers may detach and settle elsewhere in the culture flask. From above, lacunae surrounded by double cell strings which resemble acini as found in adencarcinomas could be seen. In type C cultures, the cells formed large patches of growing cells on the feeder layer and small floating spherules of viable cells.

The behavior patterns observed in culture of the four main categories of human lung carcinomas cultured directly and after passage in nude mice are summarized in Tables 1-3 and Figs. 1-5. Generally, well-differentiated carcinomas exhibited in vitro a more characteristic culture pattern than found for the poorly differentiated tumors, but most cultures could be easily characterized. It appears that cultures derived from SqCC are strongly associated with type A behavior. In cultures from AdC, type B prevails and in those from SCLC, type C is predominant. A variety of patterns was observed in cultures derived from LCC (Fig. 5).

The histological aspect of those lung carcinomas which were grown in nude mice did not differ significantly from that of the original tumor. This remained so after many passages and over periods of months or even years. The only shift in diagnosis which occurred was that from poorly differentiated SqCC to LCC or vice versa and in one LCC in the direction of AdC (RLTH 16/24). It therefore seems to be valid to lump the data from primary cultures and those derived after passing in nude
mice and to compare the cultural behavior of all human lung carcinomas investigated whether or not passaged in nude mice. The results are given in Table 3. It appears that cultural behavior is more consistent in SqCC, AdC, and SCLC than in cultures derived from LCC (Table 2). In the last group several patterns occurred, sometimes in one culture. Cloning experiments of RLTH 44/1 (AB), RLTH 49/1 (AC), and RLTH 35/1 (AC) allowed propagation in culture of each pattern separately.

According to the data presented in Table 3, a proportion of the cultures derived from human lung carcinoma xenografts in nude mice showed a heterogeneous pattern in contrast to the cultures started from fresh tumors. Cultures from LCC showed a behavior pattern of either one of the three types mentioned above or a combination of these. Five of such LCC subcultures have presented a stable pattern so far during eight months of culture, three of type A as in SqCC. One of the LCC showed two types from the start, namely types A and B or C which could be separated by cloning. One culture of a LCC (RLTH 16/22) was scored as type A at earlier passages, but cultures derived from passage 22 also produced typical type B and type...
Fig. 4. Histological appearance and pattern of in vitro cultures of human SCLC. I, histological appearance of a SCLC (RLTH 77/pt). Area of tumor cells with scant to moderate amount of cytoplasm and round to oval nuclei showing variable hyperchromasia and characteristic stippled chromatin. Note intensive hematoxylin staining of vascular walls due to presence of DNA (lower right). Hematoxylin-phloxine-saffron × 440. II, darkfield overview of a primary SCLC (RLTH 77/pt) culture 2 weeks after plating. Many small cells have moved out of the tumor piece. × 110. III, characteristic C type spherules of a SCLC culture (RLTH 84/3) 16 h after plating, well attached to the feeder layer. These spherules, as well as the floating ones, can be distinguished from those in a SqCC culture by the cobblestone aspect of the surface and by the absence of a halo. × 72. IV, higher magnification of III showing cobblestone aspect of the surface in more detail. × 460. V, 2 days after plating spherules from SCLC (RLTH 84/3) are firmly attached to the feeder layer and cells move out and spread at random. Several dividing cells can be seen. × 192. VI, 7–10 days after plating. This SCLC culture (RLTH 77/pt) shows a “burst” of many randomly arranged, proliferating cells. Spherules no longer exist; however, they will be formed again after transfer in suspension. × 150.

C groups. The histological picture of the 24th passage in nude mice of this tumor showed papillary structures in addition to squamous cell differentiation. This in vitro differentiation pattern of RLTH 16 tumor cells is suggestive for a stem cell nature of these cells. Generally, however, the cultural behavior pattern was remarkably stable for all cultures. The same holds for the histological aspect of the many passages of these tumors in nude mice (Table 2).

DISCUSSION

At the time the experiments were started, lung cancer and carcinoma cells in general were difficult to grow in vitro in contrast to sarcoma and normal mesenchymal cells (2). This difficulty may be due in the first place to the epithelial characteristics of carcinomas, where the cells, depending on the tumor type and grade of differentiation, are more or less firmly joined
Fig. 5. Histological appearance and various patterns of in vitro cultures of human LCC of the lung. I, histological appearance of a LCC (RLTH 16/pt). undifferentiated malignant epithelial tumor containing many large cells with clear cytoplasm. Some giant nuclei can be seen. Periodic acid-Schiff stain negative. HPS × 120. III', photomicrograph of a cloned LCC culture (RLTH 44/1 clone). This cloned subline developed from one cell out of a group of cells showing type A culture pattern. Notice sharp smooth outer edge of the epithelial monolayer. Cells of this subline s.c. injected into nude mice formed a SqCC. × 370. III', groups of cells showing a B type behavior pattern in a LCC culture (RLTH 44/1) 1 week after inoculation. Irregular multilayers of viable cells are formed with streamers (out of focus). × 370. III', III', III', micrographs from different areas of one LCC (RLTH 16/22) culture. Various cell groups showing A, B, or C type culture pattern were found. The same was observed in parallel cultures. III', “outgrowth” of an originally characteristic type C spherule. It consists of a burst of cobble stones. × 370. III', center of a compact epithelial monolayer with a clear cut type A aspect. × 370. III', micrograph of strands of secreting vacuolated cells surrounding “acinar” lacunae. × 290.

to each other by desmosomes and other junctions; in addition, this difficulty may be due to the presence of connective tissue, as will be explained later. To start a monolayer or semisolid culture, it is necessary to disintegrate the tumor into single cells or cell clusters. This can be done mechanically and/or enzymatically with collagenase (7). The result is a suspension of single cells (connective tissue cells and mostly nonviable tumor cells) and cell clusters consisting mainly of viable tumor cells (Fig. 2, phase I). When the cells are cultured without a feeder layer, connective tissue cells attach to and grow on the bottom of the culture flask, while most of the carcinoma cells appear as floating cell clusters (spherules or botryoids). In fact, these cultures are very selective in favor of the growth of connective tissue cells. The very low percentage of carcinoma cells that proliferate form impenetrable sheets firmly attached to the surface. These sheets can be mechanically detached, but this is
very harmful for the cells. After transfer, they do not settle on a surface without a feeder layer but remain floating as a sheet; the tumor cells die and nonmalignant connective tissue cells continue to grow.

The feeder layer system used by us indeed acts selectively in favor of the carcinoma cells as shown by the fact that none of the later passages of the tumor cultures contained connective tissue cells. Cells of nonmalignant continuous cell strains [3T3, C3H10T½, three cloned lines of a mouse spleen line (4), two rat embryo cell lines] as well as cells from early passages of rat skin fibroblasts plated onto complete C3H10T½ feeder cell sheets died within two days. None of these immortalized cell lines or fibroblasts could be recovered. Freshly derived bone marrow cells are an exception; they grow well on feeder layers forming colonies and "cell bursts."

Rheinwald and Green (8, 9) successfully grew (malignant) teratoma cells as well as skin keratinocytes on 3T3 feeder layer cells, according to the technique introduced by Puck et al. (5). Recently, Rheinwald and Beckett (10) also grew squamous cell carcinomas of the head and neck by the same procedure. In their system, the feeder layer cells were inoculated along with the tumor material. The number of feeder layer cells was such that they could cover only one-third of the surface. Normal cells present in the tumor material could grow directly on the surface of the flask. This makes it, at least in the earlier passages, necessary to differentiate between growing tumor cells and nonmalignant cells, which is quite difficult. In our system, the surface is totally covered with feeder cells before tumor material is inoculated. This prevents nonmalignant cells from attaching directly to the plastic surface and thus from growing.

After attachment onto the feeder layer nonmalignant cells die within two days. An additional advantage of our method especially in the case of SqCC is that the tumor cells growing on feeder layer can be removed from the culture flask without loss of viability, which is not the case when tumor cells grow directly on plastic or glass surfaces.

Carney et al., (6, 11) developed a special serum-free hormone supplemented (HITES) medium selective for the growth of SCLC cells. It is selective for SCLC because these cells have the capacity to grow in suspension. Nonmalignant cells except bone marrow derived cells have to anchor to grow and survive. Our SCLC lines also grew well in their medium. However, the non-small cell bronchial tumors did not grow in HITES medium, even after they had become immortal.

In conclusion, the feeder layer culture system has the advantage that it does not require the preparation of single cell suspensions. It may be a useful adjunct in the classification of lung carcinomas. The finding that the characteristics of the various tumors remained unchanged during prolonged passaging in culture as well as in nude mice is remarkable. The culture technique described here may offer new possibilities for in vitro testing of drug sensitivity.

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