Characterization of Two Cell Lines with Distinct Phenotypes Established from a Patient with Small Cell Lung Cancer

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ABSTRACT

Two small cell lung cancer (SCLC) cell lines were established from pericardial and pleural effusions of a patient with histopathologically proven SCLC of the oat cell type. Chemotherapy was administered without response during the 148-day period prior to the establishment of the first cell line, SCLC-22H, and some of the same drugs were administered in the 15 days prior to the establishment of the second cell line, SCLC-21H. Both cell lines differed markedly in their biochemical, kinetic, and morphological properties. Although the biomarkers L-Dopa decarboxylase, bombesin, carciinoembryonic antigen, and neurotensin were detectable in SCLC-22H, they were undetectable or low in SCLC-21H. The population doubling time was twice as fast and the colony forming efficiency higher in SCLC-21H than in SCLC-22H. They both expressed high concentrations of the SCLC markers enzymes neuro-specific enolase, the creatine kinase isoenzyme BB and showed no significant differences in their chromosomal characteristics. c-myc was amplified and expressed in both cell lines, and SCLC-21H had an additional rearranged and amplified EcoRI c-myc fragment. Morphological differences were apparent in liquid culture, cytology, and xenograft histology; SCLC-21H grew much more loosely than SCLC-22H, and had more prominent nucleoli and more abundant cytoplasm. Ultrastructurally dense core granules were present in both cell lines. SCLC-21H thus expressed properties of the variant cell type of SCLC, whereas SCLC-22H had mixed classic/variant features. An in vitro progression of the patient's tumor from a pure small cell to a mixed small cell/large cell morphology could be demonstrated, which suggests that cell line SCLC-22H represents a cell type characteristic for the transitional phase of the tumor. The features of this cell line therefore define a new subclass of SCLC called transitional cell type. SCLC-22H may be of use to study the mechanisms involved in the classic to variant transition, which probably has a considerable impact on the prognosis and response to therapy.

INTRODUCTION

Recent in vitro studies performed on 5 permanent human SCLC cell lines established at the National Cancer Institute, Bethesda, MD by Carney et al. (7) and Gazdar et al. (2) resulted in a subclassification of SCLC into classic and variant cell types based on the expression of neuroendocrine properties. The authors found that the majority of their cell lines (70%) had expressed properties of the variant cell type of SCLC, whereas SCLC-22H had mixed classic/variant features. An in vitro progression of the patient's tumor from a pure small cell to a mixed small cell/large cell morphology could be demonstrated, which suggests that cell line SCLC-22H represents a cell type characteristic for the transitional phase of the tumor. The features of this cell line therefore define a new subclass of SCLC called transitional cell type. SCLC-22H may be of use to study the mechanisms involved in the classic to variant transition, which probably has a considerable impact on the prognosis and response to therapy (30%), however, lacked DDC and bombesin, and 10 of 15 of these cell lines had a more large cell-like morphology; these were termed variant SCLC cell lines.

Further studies revealed additional differences between both subclasses of SCLC: (a) classic cell lines had higher levels of NSE than variant cell lines (8) and detectable levels of neurotensin (9) and CEA (10); (b) the PDT in liquid culture and the latent period of nude mouse xenografts were shorter and the colony forming efficiency in soft agarose higher for variant cell lines than those for classic cell lines; (c) variant cell lines had a more than 5-fold amplification of the c-myc protooncogene; whereas classic cell lines, with one exception, were not amplified (2, 11); (d) variant cell lines were resistant to radiotherapy but sensitive to treatment with interferon (12, 13); and (e) classic cell lines had single or clustered dense core granules on electron microscopy and only 2 of 6 variant cell lines had single dense core granules (1). Both SCLC subclasses, however, had equal amounts of CK-BB (14) and a chromosomal 3p deletion (15), which clearly distinguished them from non-SCLC. A summary of the respective properties is given in Table 1.

At the time of diagnosis approximately 6–18% of patients with SCLC have a mixed small cell/large cell morphology (16, 17). This tumor type is comparable to pure SCLC with respect to age and sex distribution, tumor spread, and sites of metastases at the time of diagnosis. However, patients with mixed small cell/large cell tumors have a considerably worse prognosis and lower treatment response rate than those with pure SCLC (18). Variant SCLC cell lines are believed to be the in vitro correlate of the mixed in vivo small cell/large cell tumors. The original tumor histology of the patients from whom the 50 SCLC cell lines were derived revealed in 5 cases a mixed small cell/large cell morphology (2). Cell lines established from these patients belonged to the variant subclass of SCLC with large cell-like morphology. The remaining 10 variant cell lines (5 large cell-like morphology) were derived from patients with pure small cell tumors. Three of these cell lines belonged to the classic subclass during early in vitro cultivation and converted to the variant type after prolonged cultivation or xenotransplantation (one with large cell-like morphology). The remaining 4 cell lines with biochemical and morphological characteristics of the variant SCLC subclass derived from patients with pure small cell tumors could have been a result of clonal selection during early cultivation.

Amplification and expression of the c-myc protooncogene was with one exception (NCI-N231) exclusively found in variant SCLC cell lines with large cell-like morphology (8 of 11). This was in contrast to the amplification and expression of N-myc (19) and L-myc (20) and suggested that c-myc might be responsible for the altered morphology of this SCLC subclass. In a recent study, Johnson et al. (21) showed that a cell line with classic features altered its morphology and growth properties but not its biochemical characteristics into those of the variant subclass upon transfection and expression of c-myc.

Although ample evidence has been provided for the presence of at least 2 SCLC subclasses, classic and variant with and
from the right middle lobe of the lung (Fig. 1A), the pericardium (Fig. 2D), the liver (Fig. IF) revealed a typical mixture of small and large cell-like elements with a dominance of the latter and no apparent proportional differences in the individual specimens.

Large cell-like elements with a dominance of the latter and no apparent proportional differences in the individual specimens.

MATERIALS AND METHODS

Patient and Cell Lines. A male Caucasian patient, age 45, was admitted to the hospital with unproductive cough, hoarseness, and a mediastinal enlargement. Tissue obtained by mediastinoscopy on October 10, 1981, revealed pure SCLC of the oat cell type (Fig. 1A), and tumor metastases were found in the liver and bone marrow. The first chemotherapy cycle consisted of ifosfamide, etoposide, and vindesine and the tumor cytology showed typical small cell elements interspersed with larger cells which had more cytoplasm and prominent nucleoli. As a result cyclophosphamide, doxorubicin, and vincristine. Fifteen days later, on March 12, 1982, a left sided pleural effusion was punctured which contained an abundance of larger cells which had more cytoplasm and prominent nucleoli with larger cells which had more cytoplasm and prominent nucleoli. This specimen gave rise to cell line SCLC-22H. The patient and the tumor remained asymptomatic.

Morphological Examinations. Tumor tissue obtained by biopsy, autopsies, and from nude mouse xenografts was performed by fixation in a formaldehyde-glutaraldehyde-picric acid fixative (23), postfixation in OsO4, embedding in Epon, and staining with uranyl acetate-lead citrate. Morphological studies. Kinetics in liquid culture were done in R10 medium, as previously described (22), results plotted on a semilogarithmic scale, and the PDT calculated from the slope of the growth curve during logarithmic growth phase. Cloning of the cells in a 0.3% agarose-containing top layer over a 0.5% agarose-containing base layer in R10 medium and s.c. xenotransplantation into NMRI athymic nude mice (nu/nu) were performed as earlier published (22).

Biomarker Evaluations. Activities and concentrations of the enzymatic markers DDC, NSE, CK, and CK-BB and concentrations of CEA were determined in homogenates of approximately 10⁷ cells in logarithmic growth phase as published (10). Radioimmunoassay kits and enzyme immunoassay kits were obtained from Pharmacia, Uppsala, Sweden for NSE, from Institut National des Radioelements, Fleurus, Belgium for CK-BB, and from Abbott, Irving, TX for CEA. Protein concentrations were measured by means of a Coomassie Brilliant Blue binding protein assay (Bio-Rad, Munich, West Germany).

The concentrations of the 14-amino acid amphibian peptide bombesin and the 13-amino acid neuroendocrine peptide neurotensin were evaluated in acetic cell extracts. Cells collected in logarithmic growth phase were washed twice in PBS, made shear in 2 m acetic acid, boiled in a water bath for 10 min, and centrifuged at 10,000 × g and 4°C for 15 min. Supernatants were lyophilized and dissolved in PBS supplemented with 0.25% w/v bovine serum albumin. Commercial polyclonal double antibody radioimmunoassays were applied as described by the manufacturer (Immuino Nuclear Corporation, Stillwater, MN) and protein concentrations were determined according to the method of Lowry et al. (24). The radioimmunoassay for bombesin cross-reacts with the mammalian bombesin analogue gastrin releasing peptide, a 27-amino acid neuroendocrine peptide with comparable biological activities. The detection limits were 50 fmol bombesin and 10 fmol neurotensin/ml, respectively.

Cytogenetic Analyses. To examine the cell lines for modal chromosome numbers, chromosomal aberrations, and the presence of HSRs and DMs, metaphase chromosome spreads were prepared by a modified version of the method described by Jaeger and Kuhn-Schlage (25) as follows. Cell aggregates were disrupted by trituration and the culture medium replaced by fresh medium containing 15 IU/ml heparin (Hoffmann-La Roche, Grenzach-Wyhlen, West Germany). Two h later 5 µg/ml ethidium bromide (Serva, Heidelberg, West Germany) was added, incubated for 1.5 h, and then 40 ng/ml Colcemid (GIBCO) were added followed by an additional 30-min incubation period at 37°C. Mitoses were collected, chromosome spreads were prepared, and G-banding performed by the trypsin-Giemsa technique (26). For each cell line 50 metaphases were evaluated for chromosome numbers and 9–10 for chromosomal aberrations.

DNA and RNA Analyses. Cell lines were harvested during log growth phase and DNA and RNA prepared by the methods of Hieter et al. (27) and Chirgwin et al. (28), respectively. Ten µg of DNA from different SCLC cell lines were digested with EcoRI and Southern blots were performed (29). Ten µg of total RNA were electrophoresed in 0.8% agarose and Northern blots prepared. Gene probes used for evaluation of the myc family of genes included a Smal-EcoRI fragment for L-myc (20), an NB-1 fragment for N-myc (30), and a ClaI-EcoRI fragment for the third exon of c-myc (31). Blots were hybridized to L-, N-, and c-myc probes, washed as reported by Little et al. (11) and Nau et al. (19, 20), and autoradiographed.

RESULTS

Morphological Examinations. The gross morphological appearances of both cell lines, SCLC-22H and SCLC-21H, are compared in Fig. 2, A and B. SCLC-22H, the first cell line established, grew suspended as tight amorphous aggregates, whereas SCLC-21H, the second cell line established, grew as

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Properties of classic and variant SCLC cell lines</th>
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<tr>
<td>Property</td>
<td>Classic</td>
</tr>
<tr>
<td>Morphology</td>
<td>Small cell</td>
</tr>
<tr>
<td>Dense core granules</td>
<td>Clusters/single</td>
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<tr>
<td>t-Dopa decarboxylase</td>
<td>Present/high</td>
</tr>
<tr>
<td>Bombesin-like immunoreactivity</td>
<td>Present/high</td>
</tr>
<tr>
<td>Carcinomembronyc antigen</td>
<td>Present/high</td>
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<td>Neurotensin-like immunoreactivity</td>
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<tr>
<td>Neuron-specific enolase</td>
<td>High</td>
</tr>
<tr>
<td>Creatine kinase</td>
<td>High</td>
</tr>
<tr>
<td>Population doubling time</td>
<td>Long</td>
</tr>
<tr>
<td>Colony forming efficiency</td>
<td>Low</td>
</tr>
<tr>
<td>Xenotransplant latent period</td>
<td>Long</td>
</tr>
<tr>
<td>Sensitivity to radiation</td>
<td>High</td>
</tr>
<tr>
<td>Sensitivity to interferon</td>
<td>Absent</td>
</tr>
<tr>
<td>c-myc amplification</td>
<td>Absent</td>
</tr>
<tr>
<td>c-myc overexpression</td>
<td>Rare</td>
</tr>
<tr>
<td>Chromosome deletion 3p (14-23)</td>
<td>Present</td>
</tr>
</tbody>
</table>

without large cell-like morphology, and for the occurrence of spontaneous and induced transitions of classic cell lines to variant types, it is yet unclear whether such a transition can occur in vivo or if the different subclasses of SCLC coexist from the time of tumor development. In this paper we describe the characterization of 2 SCLC cell lines started from different metastatic sites within a period of 15 days from the same patient having distinct biochemical, morphological, and kinetic features. The possibilities of an in vivo phenotypical transition from the classic to the variant subclass of SCLC are discussed, and a new subclass of SCLC called transitional cell type is introduced.
Fig. 1. Histological and cytological characteristics of the patient's tumor at different time points. A, mediastinal specimen at the time of diagnosis showing SCLC of the oat cell type; B, pericardial effusion collected 148 days later that gave rise to cell line SCLC-22H with typical small cell elements interspersed with larger cells; C, pleural effusion collected 15 days later that gave rise to cell line SCLC-21H showing predominantly large cells with visible nucleoli; D–F, autopsy specimens obtained 6 days later from the right middle lobe of the lung (D), the pericardium (E), and the liver (F) with a typical mixture of small and large cell-like elements. H & E, ×680.
Fig. 2. Morphological characteristics of cell lines SCLC-22H and SCLC-21H. A and B, gross morphology of SCLC-22H at passage 22 growing as tight amorphous aggregates (A) and SCLC-21H at passage 16 growing as sheets and chains (B) in liquid culture. Phase contrast, ×340. C and D, cytology of SCLC-22H at passage 13 showing a mixture of cells with small and large cell-like features (C) and SCLC-21H at passage 15 showing almost exclusively large cell-like features (D). H & E, ×860. E and F, histology of nude mouse xenografts of SCLC-22H at passage 16 showing SCLC of the intermediate cell type with few large cell-like elements (E) and SCLC-21H at passage 8 showing predominantly large cell-like elements (F). H & E, ×340.
PROGRESSION OF SCLC

![Graph showing growth curves of cell lines SCLC-22H at passage 18 (•) and SCLC-21H at passage 11 (○).](image)

Fig. 3. Growth curves of cell lines SCLC-22H at passage 18 (•) and SCLC-21H at passage 11 (○). Cells were grown in R10 medium, and the cell concentration was determined at the indicated time intervals. Bars, range of cell concentrations in duplicate samples; symbols, mean cell concentration. d, days.

SCLC-21H had absent or low concentrations of DDC, CEA, bombesin, and neurotensin and had lower levels of NSE than did SCLC-22H which defined this cell line from biochemical aspects as a variant SCLC cell line. DDC activities ranged from 0–20 units/mg and CEA levels from <2–6.5 ng/mg up until passage 16. Thereafter, both marker concentrations steadily increased and were 267 units/mg for DDC and 108 ng/mg for CEA at passage 146. CK activities and CK-BB levels were persistently high in both cell lines.

**Cytogenetics and Oncogene Analyses.** The modal chromosome numbers were 43 for cell line SCLC-22H with a range from 40–47 and 42/43 for cell line SCLC-21H with a range from 39–44 (Fig. 4). DMs were found in 1 of 50 metaphases and HSRs in 1 of 10 metaphases located on chromosome 2q of cell line SCLC-22H. In contrast, neither DMS nor HSRs were present in cell line SCLC-21H. Both cell lines had a deletion 14–23 of the short arm of at least one chromosome 3 as previously described by Whang-Peng et al. (15). This deletion was found in 4 of 10 metaphases of SCLC-22H and 5 of 9 metaphases of SCLC-21H. Common chromosomal abnormalities indicating a common derivation of both cell lines were (a) marker chromosomes 1 (SCLC-22H, 8 of 10 metaphases; SCLC-21H, 7 of 9 metaphases) and 2 (SCLC-22H, 7 of 10 metaphases; SCLC-21H, 9 of 9 metaphases); (b) deletion 6q (SCLC-22H, 10 of 10 metaphases; SCLC-21H, 5 of 9 metaphases); (c) aberration 12,1 (SCLC-22H, 8 of 10 metaphases; SCLC-21H, 9 of 9 metaphases); and (d) aberration 12,2 (SCLC-22H, 8 of 10 metaphases; SCLC-21H, 8 of 9 metaphases).

Structural aberrations of chromosomes 1, 2, and 8, which are known to contain the L-, N-, and c-myc genes, respectively (20–23), could not be identified. Representative metaphase spreads for both cell lines are depicted in Fig. 5.

Fig. 6 shows the results of DNA and RNA analyses of cell lines SCLC-22H, SCLC-21H, and control cell lines with a third exon c-myc probe (31). Both cell lines were amplified for the 12.7-kilobase germ line EcoRI c-myc fragment at levels comparable to control cell line NCI-N417 (about 50-fold). Cell line SCLC-22H had an additional, rearranged, and amplified c-myc band that was not detectable in other SCLC cell lines studied including SCLC-22H. Ten µg of total cytoplasmic RNA were analyzed by Northern blotting, and a single 2.3-kilobase band, the normal size of the c-myc transcript, was found. The c-myc mRNA levels of cell lines SCLC-22H and SCLC-21H were between those of cell lines NCI-H146 (relative amount of c-myc mRNA, 6-fold) and cell line NCI-N417 (relative amount of c-myc mRNA, 25-fold). The amount of c-myc mRNA of cell line SCLC-21H appears to be more than the amount of SCLC-22H in the blot shown in Fig. 6. This result, however, could

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**Table 2. Properties of cell lines SCLC-22H and SCLC-21H**

<table>
<thead>
<tr>
<th>Property</th>
<th>SCLC-22H (passage no.)</th>
<th>SCLC-21H (passage no.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Small cell/large cell-like</td>
<td>Large cell-like</td>
</tr>
<tr>
<td>Dense core granules</td>
<td>Single (23)</td>
<td>Single (18)</td>
</tr>
<tr>
<td>L-Dopa decarboxylase</td>
<td>300 units/mg (10)</td>
<td>&lt;1 unit/mg (13)</td>
</tr>
<tr>
<td>Bombesin-like immunoreactivity</td>
<td>470 fmol/mg (10)</td>
<td>&lt;50 fmol/mg (12)</td>
</tr>
<tr>
<td>Carcinoembryonic antigen</td>
<td>80 ng/mg (14)</td>
<td>&lt;2 ng/mg (16)</td>
</tr>
<tr>
<td>Neurotensin-like immunoreactivity</td>
<td>25 fmol/mg (24)</td>
<td>&lt;10 fmol/mg (25)</td>
</tr>
<tr>
<td>Neuro-specific enolase</td>
<td>661 ng/mg (9)</td>
<td>598 ng/mg (9)</td>
</tr>
<tr>
<td>Creatine kinase activity</td>
<td>4,353 millimlits/mg (10)</td>
<td>2,929 millimilits/mg (9)</td>
</tr>
<tr>
<td>Creatine kinase-BB level</td>
<td>11,479 ng/mg (10)</td>
<td>6,338 ng/mg (9)</td>
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<tr>
<td>Population doubling time</td>
<td>75 h (18)</td>
<td>45 h (11)</td>
</tr>
<tr>
<td>Colony forming efficiency</td>
<td>2.2% (14)</td>
<td>3.4% (15)</td>
</tr>
<tr>
<td>c-myc amplification</td>
<td>Present (21)</td>
<td>Present (15)</td>
</tr>
<tr>
<td>c-myc rearrangement</td>
<td>Absent (21)</td>
<td>Present (15)</td>
</tr>
<tr>
<td>c-myc expression</td>
<td>High (21)</td>
<td>High (15)</td>
</tr>
<tr>
<td>Chromosome deletion 3p</td>
<td>Present (20)</td>
<td>Present (7)</td>
</tr>
</tbody>
</table>

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of the myc family, i.e., N- and L-myc, were neither amplified nor expressed in both SCLC cell lines. Other genes of c-myc mRNA in both cell lines are comparable. Other genes of the myc family, i.e., N- and L-myc, were neither amplified nor expressed in both SCLC cell lines.

**DISCUSSION**

We established 2 SCLC cell lines from specimens of different metastatic sites collected from the same patient within a period of 15 days. Both cell lines (designated SCLC-22H and SCLC-21H) had distinct biochemical, genetic, and morphological properties (Table 2). At diagnosis the patient's tumor revealed pure oat cell morphology, but treatment was unsuccessful. The second tumor specimen obtained (pericardial effusion) showed morphological features of a mixed small cell/large cell tumor and the resulting cell line (SCLC-22H) had morphological features of the classic and variant cell type, biochemical and kinetic features of the classic cell type, and molecular biological features of the variant cell type. The third tumor specimen obtained (pleural effusion) had more obvious small cell/large cell features, and the resulting cell line (SCLC-21H) exhibited all properties of the variant cell type. At autopsy the patient's tumor had converted into a mixed small cell/large cell tumor with a dominance of large cell-like elements in all metastases examined.

The presence of a heterogeneous stem cell population in established SCLC cell lines has been demonstrated by Olsson et al. (35) and Spang-Thomsen et al. (36) with regard to differences in nude mouse tumorigenicity, colony forming efficiency, reactivity with monoclonal antibodies, and radiosensitivity. Other authors, however, did not find any significant differences in the aforementioned properties among subclones of established SCLC cell lines (2, 37).

Multiple cell lines of SCLC origin established from different metastatic sites also did not differ markedly in their morphological appearances (38), expression of biomarkers, and reactivity with monoclonal antibodies (37). The data presented here are to our knowledge the first describing a difference between SCLC cell lines established from the same patient.

Transitions of classic SCLC cell lines to the variant phenotype after prolonged cultivation (months to years) or xenotransplantation have been published (2, 10, 11, 39). These changes were observed in 6 cell lines (NCI-H60, NCI-N179, NCI-H289, NCI-N417, OH-1, SCLC-16H) in which all had lost neuroendocrine properties, i.e., DDC activities and dense core granules, and some also had altered their light microscopical features from a typical small cell to a large cell-like morphology. The differences between the cell lines described herein almost certainly occurred in vivo, because morphological changes were noted between the pericardial and pleural metastases and because all other differences were detected within the first 25 passages of in vitro culture. The role of cytotoxic drug therapy in the induction of this transition is unknown.

In our opinion, the patient's tumor may have slowly altered its whole biological behavior from one characteristic for the classic cell type to one characteristic for the variant cell type. Cell line SCLC-22H was established during the transitional phase, which would account for its mixed classic/variant properties, and cell line SCLC-21H was established after the transition and thus expressed all variant properties. The observed biochemical instability of SCLC-21H, i.e., absent or low concentrations of DDC and CEA during the first 16 passages and steadily increasing concentrations thereafter, may be an indication of an unstable, not yet genetically fixed state of this transition. From our results, the presence of heterogeneous stem cell populations within the patient's tumor at any time during tumor development and progression can neither be assumed nor excluded, since the described cell lines were established at different points in time from different metastatic sites. The presence of 2 or more different stem cell populations within one or both cell lines is unlikely, because SCLC-22H did not alter its biochemical properties during 65 passages and SCLC-21H has been increasingly expressing classical biochemical features in culture. A simultaneous presence of classic and variant stem cell populations, however, would result in an exponential loss of classical features because of the growth advantage of the variant SCLC cell type. There are at least two possible explanations for the presence of a rearranged c-myc gene in SCLC-21H that could not be detected in SCLC-22H. Either there are in fact 2 or more simultaneous stem cell populations in the patient's tumor without selection advantage for either one, or this rearrangement occurred during the time that elapsed between the initiation of both cell lines and thus may be associated with the transition. The rearranged c-myc band is an unlikely candidate to account for the transition, since there is no evidence of transcription. Other genes involved in this rearrangement could, however, be identified and provide a clue for the biological behavior of SCLC.

A transition from the classic to the variant subclass of SCLC is accompanied by profound changes in morphology, growth characteristics, partial loss of neuroendocrine properties, and development of radioresistance. These changes may be caused by an increased c-myc expression (21) and may involve one or more intermediate steps as depicted schematically in Fig. 7. This hypothesis would also explain why most investigators have not been able to demonstrate significant biological differences among subclones initiated from established SCLC cell lines and from different metastatic sites (3, 36, 37), since due to a gradual transition, major differences in the biology of various simultaneously picked stem cells cannot be anticipated.

Recently, Wong et al. (40) demonstrated that c-myc amplification is present in approximately 5% (2 of 45) of SCLC specimens directly obtained from the patients. An association between c-myc amplification and the tumor histology in vivo, however, could not be detected, and all 6 tumors exhibiting the mixed small cell/large cell morphology were not amplified for the c-myc gene. In addition, these authors could demonstrate a homogeneous distribution of c-myc amplification among various metastatic sites of patients. These data confirm the relative homogeneity of SCLC postulated in the present paper. It may be possible that tumors with in vivo c-myc amplification exhibit the variant phenotype if cultivated. This would account for the loose association between in vivo tumor histology and in vitro
Fig. 5. Karyotypes of representative metaphases of cell lines SCLC-22H at passage 20 and SCLC-21H at passage 7 which have modal chromosome numbers of 43 and 42, respectively. Chromosomes with aberrations that could be karyotyped are mapped at the respective chromosome number, and marker chromosomes are shown separately at the top. Arrows, break points.
phenotype expression outlined in the “Introduction.” Literary data, showing a down regulation of c-myc expression upon differentiation induction (41, 42) and a prevention of differentiation in the case of persistent c-myc expression (43-45), agree with the hypothesis expressed in Fig. 7 that a loss of neuroendocrine differentiation may be associated with c-myc overexpression.

To further elucidate the mechanisms involved in the transition of SCLC from the classic to the variant cell type, individual cell lines should be cloned to confirm their homogeneity, gene libraries should be prepared to identify differentially expressed genes, and biologically active substances capable of modifying the SCLC phenotype should be found.

ACKNOWLEDGMENTS

The authors are indebted to Drs. John D. Minna and Herb Oie for their technical advice and helpful discussions; to Dr. Gerhard Au Mueller for the performance of the electron microscopy; to Karin Beisenherz, Doris Gondrum, Petra Halboth, and Alfreda Simmons for their excellent technical assistance; to Silke Harnisch for her secretarial assistance; and to Anne B. Gregory-Bepler for correcting the manuscript.

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*Cancer Res* 1987;47:1883-1891.

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