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Two Small Cell Lung Cancer Cell Lines Established from Rigid Bronchoscope Biopsies

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Abstract—*Two new, good growing cell lines (GLC-8, GLC-11) have been established from biopsies of small cell lung cancer (SCLC). Tumor biopsies were procured by rigid bronchoscopy from tumor recurrences at the site of the primary lesions. Both tumors were clinically resistant to chemotherapy.*

Cytogenetic analysis revealed deletions in the short arm of chromosome 3. GLC-8 shows amplification of N-myc. Both cell lines show SCLC differentiations; neurosecrotory granules were present and the SCLC related hormones dopa-decarboxylase and creatine kinase were elevated. Both cell lines behave as so-called 'classic' SCLC cell lines.

INTRODUCTION

THE STUDY of tumor model systems contributes to an understanding of the biology of human cancer. The establishment of a large panel of different cell lines derived from human tumors will be helpful for this purpose. After the introduction of a specific serum free medium for small cell lung cancer (SCLC) [1], it became possible to establish SCLC cell lines on a more or less routine basis. The great majority of SCLC cell lines established until now has been derived from metastatic sites (pleural effusion, bone marrow and lymph nodes), because these locations are easily accessible. Only few have been derived from primary tumors obtained by a surgical resection [2].

In this report we describe the procedure to establish cell lines from biopsies of primary tumors procured by rigid bronchoscopy and two such cell lines are characterized.

MATERIALS AND METHODS

Bronchoscopy procedure

In two patients with SCLC a rigid bronchoscopy was performed under general anesthesia. Both patients were treated during 5 days with corticosteroids (prednisolone 30 mg daily) and broad spectrum antibiotics (amoxycillin), starting the day before the procedure. The bronchoscope (R. Wolf, Knittlinger, F.R.G.) had a distal cuff to facilitate artificial respiration during general anesthesia. Biopsies were taken under visual control. The first biopsy was for investigation by the pathologist, whereas a second and, if possible, third biopsy were taken for tumor cell culture.

Morphological methods

For light microscopy the tumor biopsy material was fixed in 8% formaldehyde, embedded in Epon and stained with toluidine blue. For transmission electron microscopy the tumor biopsy or, in the case of cell lines, the isolated cell pellets were incubated in 2% glutaraldehyde in phosphate-buffered saline. Postfixation was performed in 2% osmium tetroxide in phosphate-buffered saline. The material was embedded in Epon. Further preparations were according to routine procedures.

Culture procedure

The obtained biopsies were immediately put into tissue culture medium, which consisted of RPMI-1640 supplemented with 10 nM hydrocortisone, 5 μg/ml insulin, 10 μg/ml transferin, 10 nM 17-β-estradiol, 30 nM sodium selenite, 0.125 μg/ml bombesin, 10 ng/ml vasopressin, 10 μM ethanolaminephosphorylethanolamine and 1% (w/v) bovine serum albumin, according to the method of Minna *et al.* [3], and gentamicin (50 μg/ml) and amphotericin-B (2 μg/ml). Before culturing, the biopsies were mechanically brought into suspension. After occurrence of tumor cell growth, subcul-

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turing was done by normal tissue culture procedures. For the further routine culturing of these cell lines we use as a medium RPMI-1640, supplemented with 5×10^{-5} M β-mercaptoethanol, 2 mM glutamine, 1 mM pyruvate and gentamicin (50 μg/ml). The medium for GLC-8 contains 13% fetal calf serum (FCS) and for GLC-11 1%.

Characterization of cell lines

After 20 *in vitro* passages, the cell lines, obtained from histologically proven SCLC, were considered as established. The cell lines were characterized by cytology, electron microscopy, chromosome analysis, determination of dopa-decarboxylase and creatine kinase (CK) levels and by reaction with a panel of monoclonal antibodies (mabs) directed against SCLC associated antigens.

Panel of monoclonal antibodies (mabs)

A panel of mabs directed against SCLC associated antigens was used for further characterization. The mabs MOC-1, MOC-21, MOC-32, MOC-51 and MOC-52 are directed against neuro-endocrine tissue related antigens. MOC-31 is directed against an antigen present on epithelial tissues [4]. During the recent first international workshop on antigens of SCLC these mabs were clustered: MOC-1 and MOC-51 in cluster 1, MOC-21 and MOC-32 in cluster 1a and MOC-31 in cluster 3 [5].

RGE 53 (Eurodiagnostics, Apeldoorn, The Netherlands) reacts with Keratin 18 [6, 7]. Neurofilaments were detected with MNF (Eurodiagnostics). This antibody reacts with the 210 kD and 70 kD polypeptides of neurofilaments. HNK 1 is directed against Leu 7 [8]. Procedures for immunoperoxidase and indirect immunofluorescence staining have been described earlier [9, 10].

Biochemical analysis

Culture doubling time was assessed by measuring the increase of total culture DNA in time. DNA measurements were carried out according to the procedure described by Fiszer-Szafarz *et al.* [11]. Dopadecarboxylase determinations were performed according to described methods [12]. Creatinine kinase determinations and iso-enzyme analysis were performed with the CK Merckotest (Merck, F.R.G.).

Chromosome analysis

Fresh RPMI 1640-medium containing 15% FCS was given one day before harvest. As a metaphase arrester vinblastine sulfate (Lilly, Indianapolis, IN) was added to a final concentration of 20 ng per ml for the last 2–3 h. Cells were collected, treated with a hypotonic solution and fixed as described by Yu *et al.* [13]. Chromosome banding of air-dried metaphases was obtained by double staining with daunomycin and DAPI as previously described for isolated chromosomes in suspension [14].

RESULTS

Characterization of cell lines

The two SCLC cell lines are called GLC-8 and GLC-11. GLC-8 was obtained from a tumor recurrence in the right upper lobe bronchus 4 months after stopping chemotherapy. Both primary and recurrent tumor showed many tumor cells with nucleolated nuclei and more cytoplasm than usually found in SCLC. Electron microscopy, however, revealed characteristic dense-core granules and the tumor was diagnosed as SCLC (Fig. 1a). The recurrent tumor did not respond to vindesine. After radiotherapy (30 Gy) a partial remission was seen. GLC-8 started exponential growth immediately after explanting the cells into tissue culture medium and has kept a constant growth rate since then.

GLC-11 was obtained from a biopsy of a tumor recurrence in the right intermediate bronchus after chemo- and radiotherapy. The recurrent tumor did not respond to vindesine and teniposide. Light microscopy of the biopsy showed SCLC. Electron microscopy was not performed (Fig. 1b). GLC-11 showed also an initial exponential growth, but slowed down at about passage 10. After a few more passages, during which culture density was kept high, exponential growth was resumed again and stayed the same thereafter. The doubling times of the cell lines are 34 and 80 h.

Culture morphology and electron microscopical appearance of the cell lines were analyzed at about passages 34 and 28 respectively.

GLC-8 grows loosely attached (Fig. 2a) and GLC-11 grows as floating aggregates (Fig. 2b). Electron microscopy revealed in both the presence of dense core vesicles (Fig. 3a and b).

In addition, GLC-8 is characterized by the occurrence of many double minutes. GLC-8 shows amplification of N-myc. Chromosome numbers in both cell lines were hyperdiploid ranging up to about 60 per metaphase. Usually two chromosome 3 homologues were observed that differed in the length and banding pattern of their short arms. Whereas one was normal the other appeared to have a deletion always including part of band p21. Representative chromosome pairs for both cell lines are shown in Fig. 4.

The characterization of the cell lines is summarized in Table 1. Table 2 shows the antigenic features of the two cell lines as defined by a panel of mabs directed against SCLC associated antigens.

DISCUSSION

SCLC has a very poor prognosis, in untreated patients the median survival is less than 3 months from diagnosis [15]. Despite dramatic responses

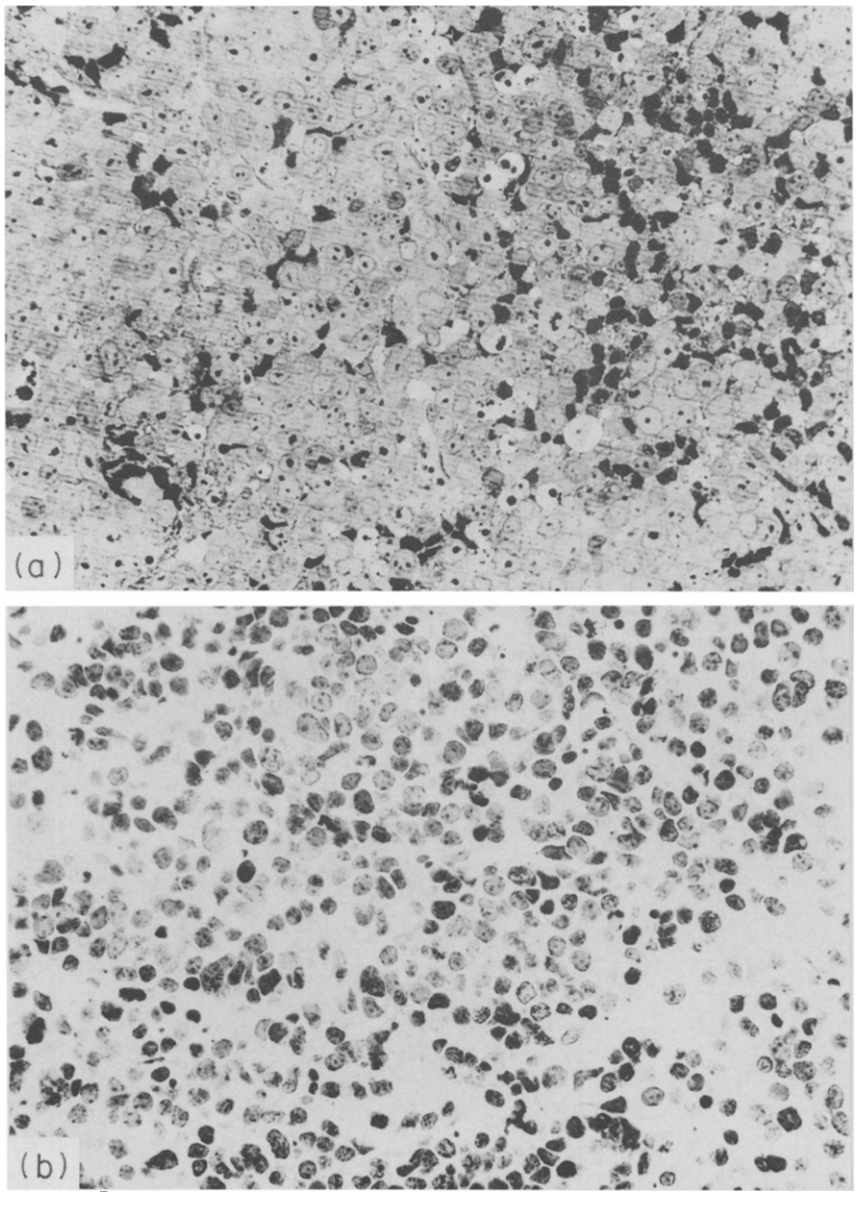


Fig. 1(*a*). *Light microscopy of Epon-embedded biopsy giving rise to GLC-8. Many tumor cells show one or more nucleoli leading to erronous diagnosis of 'large cell cancer'* (*Flores stain*, × 315). (*b*) *Light microscopy of biopsy giving rise to GLC-11; showing classic small cell cancer* (*H.E.* × 315).

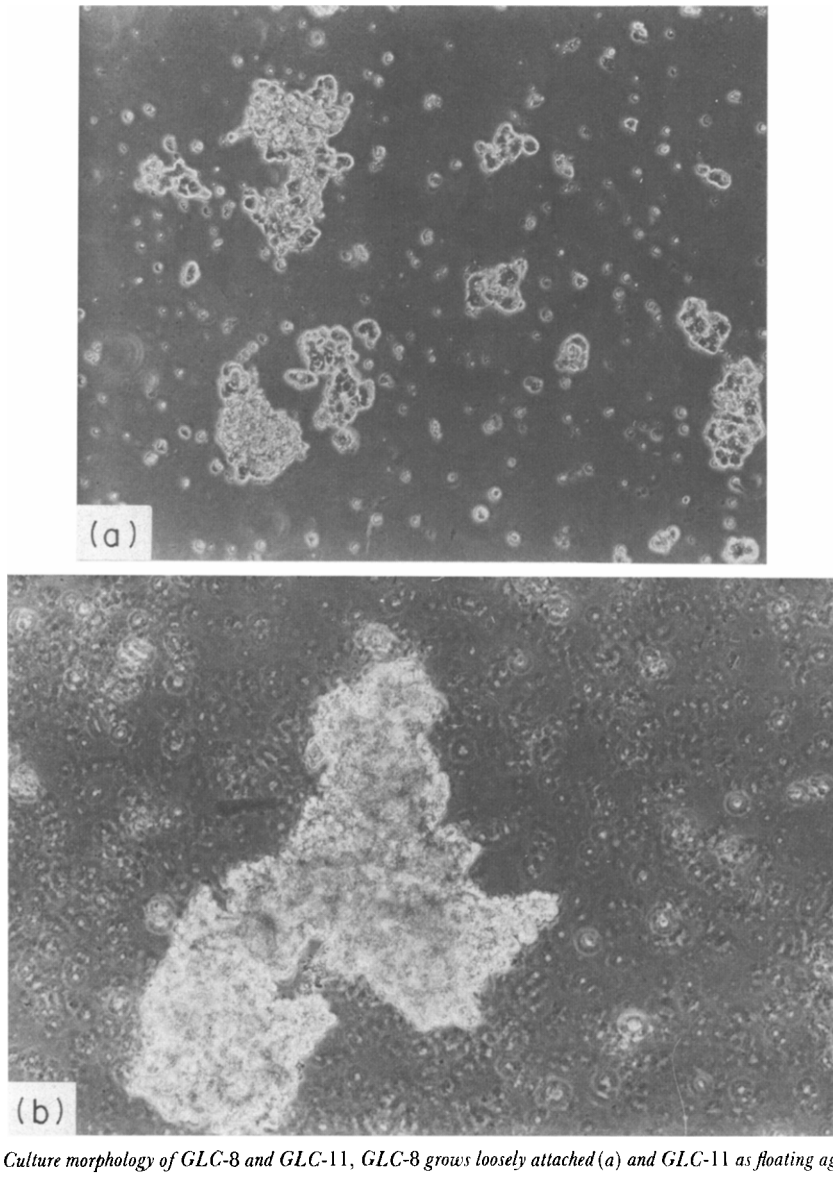
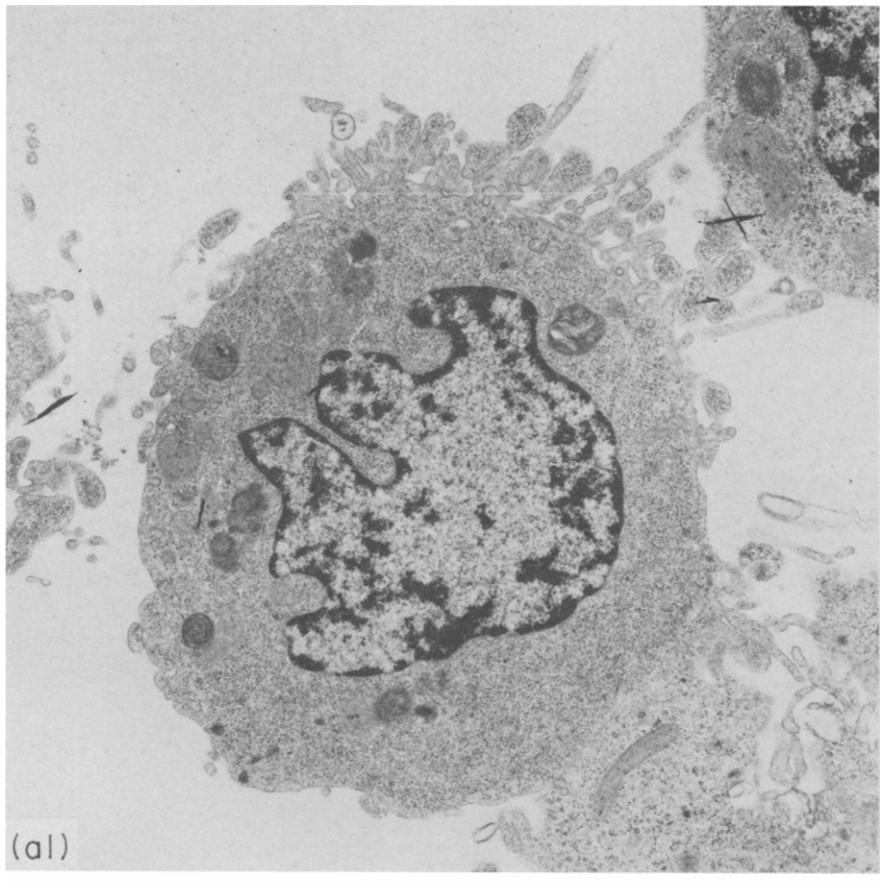


Fig. 2. Culture morphology of GLC-8 and GLC-11, GLC-8 grows loosely attached (a) and GLC-11 as floating aggregates (b).



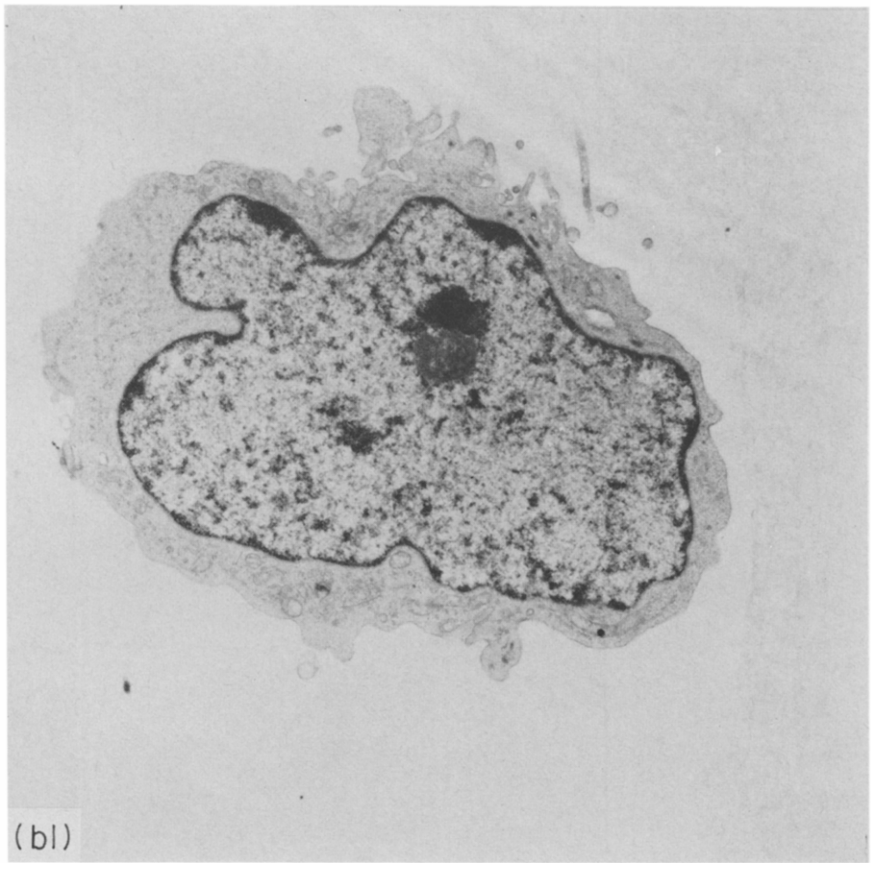
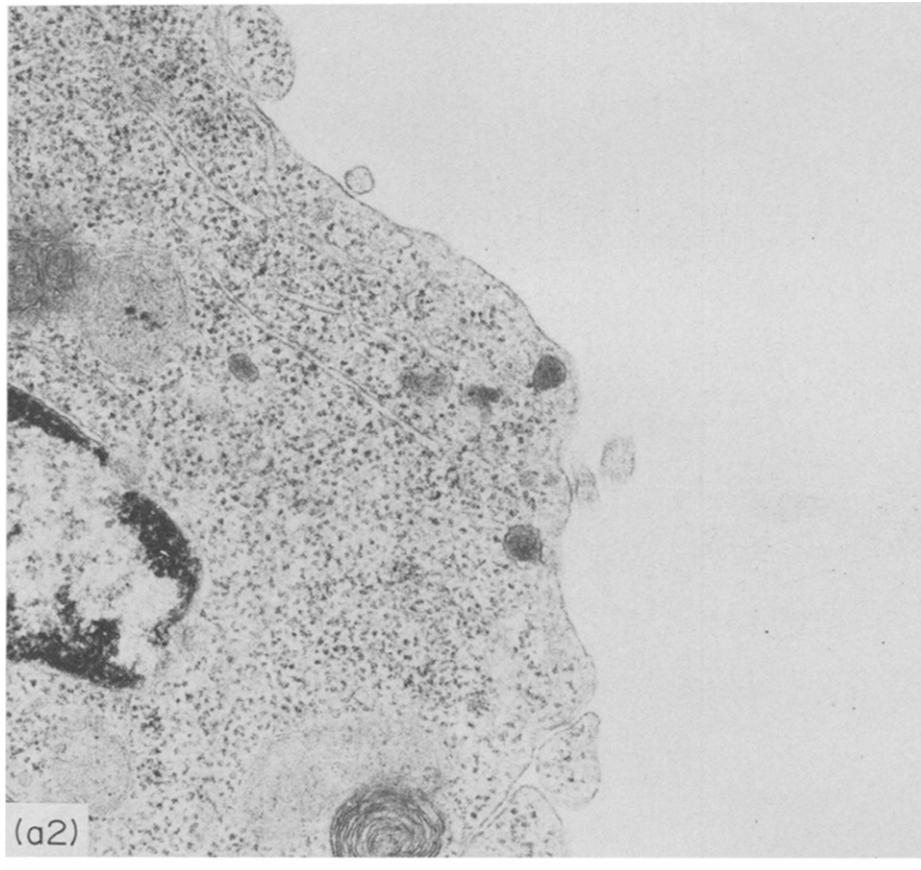


Fig. 3.



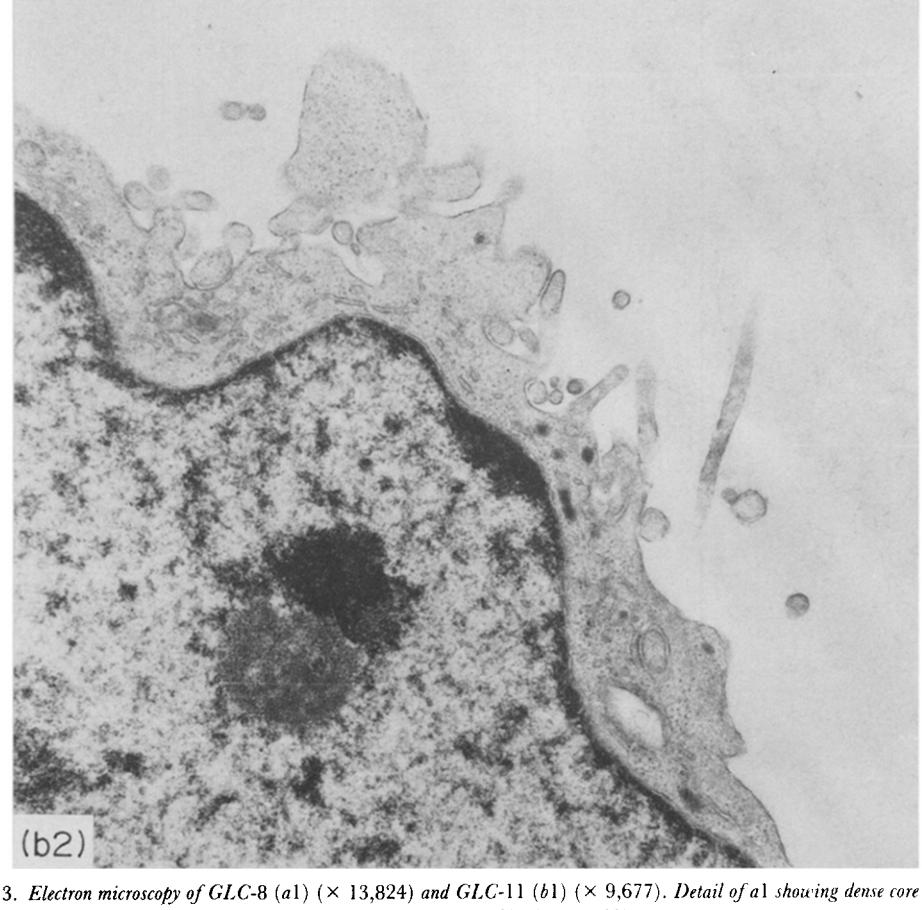


Fig. 3. Electron microscopy of GLC-8 (a1) (× 13,824) and GLC-11 (b1) (× 9,677). Detail of a1 showing dense core vesicles (a2) (× 39,398) and of b1 (× 18,939).

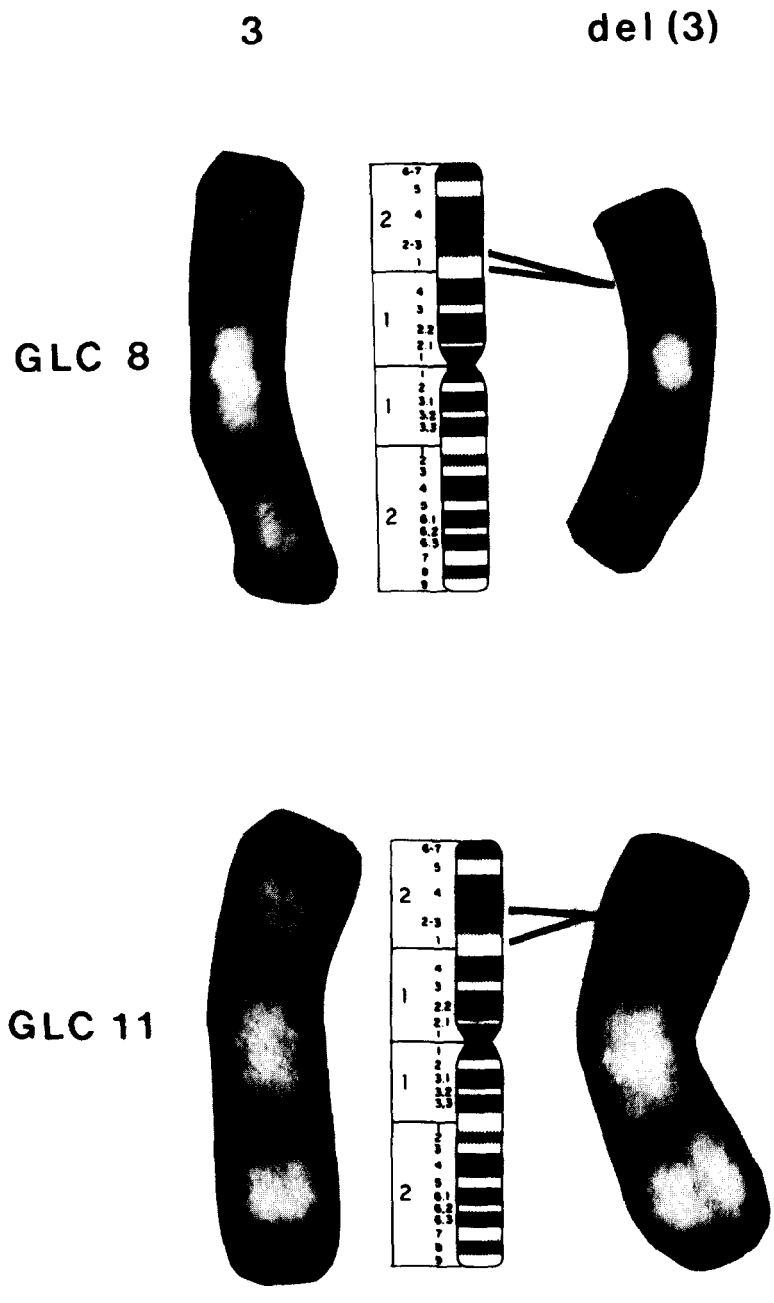


Fig. 4. Normal and abnormal homologues of chromosome 3 from metaphases of GLC-8 and GLC-11. The deleted regions are indicated in the diagram.

Table 1. Culture characteristics and enzyme content of two SCLC cell lines

	GLC-8	GLC-11
Growth in serum free culture medium	+	+
Culture doubling time	34 h	80 h
Electron microscopy dense core vesicles	+	+
Creatine kinase (units/g protein)	930	1100
Creatine kinase(%)		
BB	25	100
MB	50	0
MM	25	0
Dopadecarboxylase (units/mg protein)	100	94.4

Table 2. Reaction pattern with monoclonal antibodies

	GLC-8	GLC-11
MOC-1	++	++
MOC-21	±	++
MOC-31	+	++
MOC-32	+	+
MOC-51	+	−
MOC-52	+	+
RGE 53	75%+	−
MNF		
Neurofilaments	−	−
HNK 1	<1%+	++

only very few patients have been cured since the introduction of combination chemotherapy more than one decade ago. In most patients partial or even complete responses will be seen shortly after the start of treatment. However, tumor relapse or progression occurs eventually in almost all patients. In contrast with the initial sensitivity to cytostatic drugs, at relapse cytostatic treatment is far less effective and often fails completely [16]. The apparent reason for this failure is tumor cell resistance. The mechanisms behind the development of this *in vivo* resistance is uncertain. It might have been induced by the previous treatment, or an already initially resistant clone of a heterogeneous tumor may continue to proliferate.

The study of the already established cell line has resulted in characterization of two groups of cell lines. The so-called 'classic' lines, light microscopically resembling SCLC *in vivo*, and the 'variant' cell lines, which are 'large-cell like', resemble end-stage treatment-resistant SCLC *in vivo*, and differ in expression of some SCLC markers. It is questionable whether this culture phenomenon has any direct relation with the *in vivo* phenotype of this tumor. The neuroendocrine tissue-related dense core vesicles, as well as bombesin and dopa-decarboxylase activities, are absent in 'variant' cell lines [2]. Further investigations are necessary to understand the changes in the cell responsible for this conversion and to correlate it with the clinical situation. The two cell lines described in this report can be classified as so-called 'classic' cell lines, although both were procured from endstage SCLC and one biopsy showed a cytology more in line with changes seen in variant-type cell lines.

The presence of a deletion of the short arm of chromosome 3 has been described as characteristic for SCLC [17]. The cytogenetic analysis of the two cell lines derived from bronchoscope biopsies of primary tumors shows that they also contain the 3 p deletion. We could delimit the deletion common in both lines to band p21. In view of the inclusion of band p22 in the deletion of GLC-11, it is the distal half of band p21 which is presumably involved. This corroborates our previous cytogenetic and molecular findings in SCLC [18–20]. In GLC-8 numerous double minutes were found, a cytogenetic indication of gene amplification. Amplified genes of the myc-family have been reported to occur frequently in SCLC cell lines [18, 21, 22] either as double minutes or as specific chromosome regions, so-called homogeneously staining regions. GLC-8 shows a significant amplification of N-myc (K. Kok and C.H.C.M. Buys, unpublished results). The localization of the amplified sequences is currently under study. No amplification of C-myc, N-myc or L-myc was found in GLC-11.

Comparison of these two cell lines originating from lesions present at the site of the primary tumor with others, derived from metastatic lesions, reveals no major differences [2].

In experienced hands the establishment of SCLC cell lines is successful in about 75% of the tumor samples [2]. The majority of these cell lines have been derived from a relatively large amount of sterile tumor material. Establishing cell lines from an endobronchial tumor is probably much more difficult due to the limited size and the number of the biopsies that become available for cell culture. Furthermore, the biopsies are taken from an area known to be an ideal environment for the growth of

micro-organisms, and contamination of tumor cell culture may occur.

In our experience contamination by bacteria is rare probably due to the potent action of gentamycin in the normally used tissue culture medium. The relatively low number of successfully established cell lines from these primary lesion sites, in our hands 10% of the tumor samples, appears to reflect an intrinsic property of these tumors. Nevertheless, in carefully selected patients the success rate of tumor cell culture from bronchoscope biopsies may become high enough to open new perspectives for the study of the biology of SCLC during chemotherapy. Sequential establishment of cell lines in patients before, during and after treatment may shed more light on the processes responsible for the change from sensitive to resistant tumor.

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