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Spontaneous changes in intermediate filament protein expression patterns in lung cancer cell lines

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Summary

The usefulness of cell lines in the study and prediction of the clinical behaviour of lung cancer is still a matter of debate. However, lung tumour cell cultures have been of value in investigations concerning molecular and cell biological aspects of these neoplasms. Especially in the examination of characteristics specific for the main types of differentiation (squamous cell carcinoma, adenocarcinoma, small cell carcinoma), *in vitro* studies have been most important.

Twenty eight lung cancer cell lines were cultured for up to four years, and were examined at regular intervals for their intermediate filament protein (IFP) expression patterns using a panel of cytokeratin (CK) and neurofilament (NF) antibodies. These studies showed that the classic type of small cell lung cancer (SCLC) cell lines contain CKs 8, 18, and occasionally CK 19, while the variant-type SCLC cell lines generally express no CKs but can contain NFs. Non-SCLC cell lines, such as squamous cell carcinoma and adenocarcinoma cell lines, contain CKs 7 (in most cases), 8, 18 and 19. In one variant SCLC cell line and in one adenocarcinoma cell line CKs 4, 10 and 13, characteristic of squamous cell differentiation, were found. Although most cell lines have remained stable with respect to growth characteristics and IFP expression patterns, five lung cancer cultures exhibited a transition from one cell type to another, paralleled by changes in IFP expression. Progressions from classic to variant SCLC cell lines have been observed, next to conversions from variant SCLC to cell lines re-expressing cytokeratins. In some cases this resulted in a coexpression of CKs and NFs within a cell line and even within individual tumour cells. These results strongly support the earlier finding that CK expression in SCLC cell lines is a reliable marker for the classic type of differentiation, while the absence of CKs and the presence of NFs marks the variant type of differentiation. Our results are discussed in view of previous histological findings.

Key words: small cell lung cancer, cytokeratins, neurofilaments.

Introduction

Intermediate filament proteins (IFPs) are cytoskeletal components, which are expressed in a more or less tissue-specific fashion and are, in most cases, retained during neoplasia (Osborn & Weber, 1983; Ramaekers *et al.* 1983*c*, 1987*a*). In general, only one type of intermediate filament protein is expressed in a certain tissue or tumour. For instance, epithelial cells and carcinomas normally express only IFPs of the cytokeratin (CK) type (see, e.g., Ramaekers *et al.* 1983*d*), while neuronal cells and some neuronal tumours express only neurofilament (NF) proteins (Altmannsberger *et al.* 1984). In normal tissues, vimentin is in general found only in mesenchymal tissues.

However, in the last few years a growing number of cases has been reported, in which a coexpression of two or even three types of IFP within the same type of (tumour) cells was demonstrated. Most frequently, if coexpression occurs, vimentin is one of the IFPs present. Expression of vimentin next to CK has been found in some normal epithelial cells, such as human mesothelium and epithelia from ovarium, amnion, endometrium, thyroid gland and foetal kidney (Moll *et al.* 1983; Holthöfer *et al.* 1984; LaRocca & Rheinwald, 1984; Rheinwald *et al.* 1984; Czernobilsky *et al.* 1985; Regauer *et al.* 1985; Achtstätter *et al.* 1986). Also, in some types of primary carcinomas coexpression of CKs and vimentin is seen (Caselitz *et al.*

1981, 1984; Herman et al. 1983; Churg, 1985; Blobel et al. 1985b; Gould, 1985; Jasani et al. 1985; Upton et al. 1986), and this coexpression may also be seen in metastatic carcinoma cells in body cavity fluids (Ramaekers et al. 1983a). In carcinoma-derived cell lines the presence of vimentin is a rule rather than an exception (cf. Franke et al. 1979; Broers et al. 1985). Vimentin is expressed next to desmin in muscle cell tumours (Molenaar et al. 1985).

More recently, coexpression of other types of intermediate filaments has been reported. For instance, Brown et al. (1987), Norton et al. (1987), van Muijen et al. (1987) and Ramaekers et al. (1988) reported on the coexpression of CKs and desmin in human foetal and adult muscle tissue, and in smooth muscle tumours. Kasper et al. (1986) showed the coexistence of CK, vimentin and NF protein in human choroid plexus, while Budka (1986) demonstrated a triple expression of glial fibrillary acidic protein, vimentin and CK in papillary meningioma and renal carcinoma. Gatter et al. (1986) showed a double and triple expression of different IFPs in many lung carcinomas. Coexpression of CKs and NFs seems to be characteristic of some neuroendocrine tumours, such as neuroendocrine skin carcinomas (Merkel cell tumours; van Muijen et al. 1985; Gould et al. 1985; Moll et al. 1986), parathyroid tumours (Miettinen et al. 1985), lung carcinoids (Lehto et al. 1985; Blobel et al. 1985a; Broers et al. 1987; Ramaekers et al. 1987a), some small cell lung cancers (SCLCs; Gatter et al. 1986; Broers et al. 1987) and (poorly differentiated) squamous cell carcinomas (SQCs) of the lung (van Muijen et al. 1984; Broers et al. 1987). Also in a cell culture of a large cell carcinoma of the lung (Bergh et al. 1984), in a neuroendocrine skin carcinoma cell line (Rosen et al. 1987), in the neuroendocrine rat cell line PC12 (Franke et al. 1986) and in some SCLC cell lines (Bernal et al. 1983; Banks-Schlegel et al. 1985; Gupta et al. 1986) coexpression of CKs and NFs has been reported.

The three major types of lung cancer comprise SCLC, adenocarcinoma (AC) and SQC (W.H.O., 1982). In addition, according to the latest IASLC classification, SCLC can be subdivided into classic SCLC, variant SCLC and combined SCLC (Yesner, 1985). It is now generally accepted that lung cancer is an extremely heterogeneous type of malignancy. On the basis of detailed histological (Roggli et al. 1985), immunohistochemical (Hammar et al. 1985; Dunnill & Gatter, 1986; Broers et al. 1987, 1988) and electron-microscopic (McDowell & Trump, 1981; Trump et al. 1982; Dingemans & Mooi, 1984; Mooi et al. 1988) studies, more than one type of differentiation can be detected in the majority of lung cancers. Occasionally more than one type of differentiation can be detected in cell lines, for example in the so-called multipotent lung tumour cell cultures (Carney et al. 1984; de Leij et al. 1985). Cloned cells of these cell lines are apparently able to differentiate again into the three major types of lung cancer within the same culture. In addition, sometimes a conversion of one cell type to another has been noticed. For example, progressions of classic SCLC cell lines into the variant type of SCLC cell line have been described (Bepler et al.

1987a; Carney et al. 1984, 1985a,b; Gazdar et al. 1981, 1985; Graziano et al. 1987). Bepler et al. (1987a, 1988c) described a transitional type of SCLC with features between the classic and variant types.

Previous investigations in our laboratory on the intermediate filament expression of classic and variant SCLC cell lines have demonstrated the occurrence of CKs only in classic SCLC cell lines, while in variant SCLC cell lines no IFP or only NFs could be demonstrated (Broers et al. 1985, 1986). In recent years, cell lines in which a morphological change from one cell type to another could be seen were examined for their IFP expression patterns.

Since different CK polypeptides are expresssed in different types of lung cancer (cf. Blobel et al. 1984, 1985a; Broers et al. 1988), we paid special attention to the CK polypeptide expression of these cell lines. Using a panel of monoclonal CK antibodies, most of which are specific for one CK polypeptide, it is possible to detect SQC differentiation, AC differentiation and SCLC differentiation at the cellular level (Broers et al. 1988).

In this paper we describe spontaneous changes in intermediate filament patterns that can occur in lung cancer cell lines, especially in SCLC cell lines. In part, these alterations follow the phenotypic changes in the tumour cells, and may be correlated with recent data obtained with lung tumour tissues.

Materials and methods

Cell lines

Establishment and characterization of the cell lines used in this study have been described (Carney et al. 1985a,b; de Leij et al. 1985; Gazdar et al. 1985; Bepler et al. 1987b,c, 1988b) or will be described elsewhere. In all, 19 SCLC cell lines were investigated (NCI-H69, NCI-H128, NCI-H449, NCI-N417, NCI-H524 and NCI-H82; GLC1-M13, GLC-1, GLC-2, GLC-3, and GLC-5; SCLC-16HC, SCLC-16HV, SCLC-21H, SCLC-22H, SCLC-24H, and SCLC-86M1; NL-SCLC2 and NL-SCLC3). Next to these, one LCLC cell line (LCLC-103H), six AC cell lines, (NCI-H23, NCI-H125, GLC-A1, GLC-A2, GLC-A3, NL-AC1) and two epithelial cell lines derived from SQCs (EPLC-32M1 and EPLC-65M2) were used.

All cell lines were maintained in a humidified incubator at 37°C in 5% CO_2 and were grown in Roswell Park Memorial Institute Medium 1640 (RPMI-1640, Flow, Irvine, UK) containing Hepes, supplemented with 15% newborn calf serum (Flow). At regular intervals cells were passaged by splitting cells 1:2 to 1:3. If cells tended to attach to the culture flasks, the adhering cells were selected by removing the floating cells. As a result sublines NCI-N417-A1, NCI-N417-A2, NCI-H524-A and NCI-H82-A were obtained.

Indirect immunofluorescence technique

The procedures for the application of the indirect immunofluorescence technique have been described (Broers et al. 1986). In brief, floating cells were placed on glass slides using a Cytospin centrifuge (500 revs min^{-1} for 10 min; Shandon Southern Instruments, Astmoor, Cheshire, UK), while cells growing attached to the culture flasks were treated with 0·05% trypsin/0·02% EDTA in 0·05 M-phosphate-buffered saline (PBS), pH 7·4, and allowed to grow on glass coverslips for 1–2 days. In the first step, cells were incubated with the primary

antibody (see below) for 30–45 min. As second antibody, either fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG (1:25, Nordic, Tilburg, The Netherlands) or FITC-conjugated goat anti-rabbit IgG (1:25, Nordic) was applied to the cells for 30–45 min.

In the double-label immunofluorescence technique a mixture of two primary antibodies (one raised in mouse and one in rabbit) was applied in the first step. Cells were incubated for 30–45 min and, after washing with PBS, a mixture of FITC-conjugated goat anti-rabbit IgG and Texas Red-conjugated sheep F(ab')2 anti-mouse IgG (Amersham, Bucks, UK) with final dilutions of 1:25 and 1:50, respectively, was added. Cells were incubated for 30–45 min, and washed again. DNA was then stained by incubating the cells for 15 min with Hoechst 33258 (0·1 μg ml^{-1} in 22 mM-citric acid, 56 mM-disodium hydrogen phosphate). After washing with PBS the cells were mounted. Pictures were taken with an automatic camera, with either a 400 ASA Tri-X film (Kodak) or with a 400 ASA Ektachrome film (Kodak) for triple exposures at three different wavelengths. Overlap between the FITC and Texas Red fluorescence at the respective wavelengths was checked using preparations labelled with a single second antibody. Incubations with the second antibodies alone were used as negative controls.

Antibodies

Specificity and references of the primary antibodies used in this study are summarized in Table 1. The following primary antibodies were used.

Mouse monoclonal CK antibodies (undiluted). (1) RCK102 is a broadly cross-reacting CK antibody of the IgG1 subclass, which recognizes CKs 5 and 8, and as a result stains virtually all epithelial tissues (Ramaekers *et al.* 1987*b*). (2) RCK105 (IgG1; Ramaekers *et al.* 1987*b*) reacts only with CK 7 in immunoblotting and stains a subgroup of glandular epithelia and their tumours, next to transitional bladder epithelium and bladder carcinomas. (3) RGE53 (IgG1; Ramaekers *et al.* 1983*b*, 1985) and RCK106 (IgG1; Ramaekers *et al.* 1987*b*) are monospecific for CK 18 in immunoblotting. In general these two antibodies recognize columnar epithelial cells from digestive, respiratory and urogenital tracts, endocrine and exocrine tissues and

mesothelial cells, as well as their tumours. Generally, no reaction is found in squamous epithelia or SQCs. (4) LP2K (Lane *et al.* 1985) and BA17 (IgG1; Bartek *et al.* 1986*a,b*) stain most simple epithelia and basal cells in stratified squamous epithelia that are not keratinizing. Both antibodies recognize only CK 19 in immunoblotting assays. (5) RKSE60 (IgG1; Leigh *et al.* 1985; Puts *et al.* 1985) reacts only with keratinizing epithelial cells, and recognizes CK 10 in immunoblotting. (6) 6B10 (IgG1; van Muijen *et al.* 1986) reacts with non-cornifying squamous epithelium and with certain ciliated pseudostratified epithelia such as cylindrical epithelium of bronchi, and recognizes only CK 4 in immunoblotting. (7) Antibodies 1C7 (IgG2a; van Muijen *et al.* 1986) and 2D7 (IgG2b; van Muijen *et al.* 1986) react both with non-cornifying squamous epithelia. These antibodies recognize only CK 13 in immunoblotting studies.

NF antibodies. (1) The mouse monoclonal antibody MNF (IgG1, diluted 1:5 in PBS), is reactive with the $68 \times 10^3 M_r$ and $200 \times 10^3 M_r$ NF subunits and stains neuronal tissues and tumours (Klück *et al.* 1984). (2) The polyclonal rabbit NF antibodies pNF68 (diluted 1:400), pNF160 (diluted 1:600) and pNF200 (diluted 1:400) are specific for the three NF subunits of 68, 160 and 200 $(\times 10^3) M_r$, respectively (Nakazato *et al.* 1984), and react in neuronal tissues.

Vimentin antibodies. (1) The mononclonal antibody RV202 (IgG1; Ramaekers *et al.* 1987*b*) reacts with mesenchymal cells. (2) The polyclonal antibody pVIM prepared against bovine lens vimentin (Ramaekers *et al.* 1983*c*) also reacts with mesenchymal cells.

Antibodies RCK102, RCK105, RGE53, RKSE60, MNF, 6B10, 1C7, 2D7, and pVIM are available from Euro-Diagnostics B.V. (Apeldoorn, The Netherlands).

Gel electrophoresis and immunoblotting

For gel electrophoresis and immunoblotting experiments the same procedures were followed as described (Broers *et al.* 1986, 1988). For immunoblotting the following antibodies were used: RCK102 (undiluted supernatant), RCK105 (undiluted supernatant), RCK106 (undiluted supernatant), LP2K (diluted 1:10) and pNF68 (diluted 1:5000).

Table 1. *Specificity of antibodies used in this study*

Antibody	Mouse Ig subclass (dilution for immunofl.)	Antigen recognized	Tissue specificity	Reference
RCK102	IgG1 (undil.)	Cytoker. 5+8	Nearly all epithelial tissues	Ramaekers *et al.* (1987*b*)
RCK105	IgG1 (undil.)	Cytoker. 7	Some glandular epithelia	Ramaekers *et al.* (1987*b*)
RGE53	IgG1 (undil.)	Cytoker. 18	Glandular epithelia, not with squamous cell epithelia	Ramaekers *et al.* (1983*b*, 1985)
RCK106	IgG1 (undil.)	Cytoker. 18	Glandular epithelia, not with squamous cell epithelia	Ramaekers *et al.* (1987*b*)
LP2K	(undil.)	Cytoker. 19	Most simple epithelia	Lane *et al.* (1985)
BA17	IgG1 (undil.)	Cytoker. 19	Most simple epithelia	Bartek *et al.* (1986*a,b*)
RKSE60	IgG1 (undil.)	Cytoker. 10	Keratinizing epithelial cells	Leigh *et al.* (1985); Puts *et al.* (1985)
6B10	IgG1 (undil.)	Cytoker. 4	Non-cornifying squamous epithelia, pseudostratified epithelia	van Muijen *et al.* (1986)
1C7	IgG2a (undil.)	Cytoker. 13	Non-cornifying squamous epithelia	van Muijen *et al.* (1986)
2D7	IgG2b (undil.)	Cytoker. 13	Non-cornifying squamous epithelia	van Muijen *et al.* (1986)
MNF	IgG1 (1:5)	NF 68K, 200K	Neuronal tissues	Klück *et al.* (1984)
pNF68	Rabbit (1:400)	NF 68K	Neuronal tissues	Nakazato *et al.* (1984)
pNF160	Rabbit (1:600)	NF 160K	Neuronal tissues	Nakazato *et al.* (1984)
pNF200	Rabbit (1:400)	NF 200K	Neuronal tissues	Nakazato *et al.* (1984)
RV202	IgG1 (undil.)	Vimentin	Mesenchymal tissues	Ramaekers *et al.* (1987*b*)
pVim	Rabbit (1:25)	Vimentin	Mesenchymal tissues	Ramaekers *et al.* (1983*b*)

p, polyclonal (rabbit) antiserum. NF, neurofilaments; K, $10^3 M_r$.

Flow cytometry

The preparation of cell samples for flow cytometric analyses has been described (Ramaekers et al. 1986). Briefly, cells were centrifuged at room temperature (5 min, 500 g) and the supernatant was discarded, but fluid sticking to the wall of the tubes was allowed to cover the pellet. Cells were resuspended in this fluid by vigorously vortexing, cold ($-20°C$) 70 % ethanol was added, and the cells were kept in this solution until staining. The final cell concentration was about 10^6 cells per ml of 70 % ethanol. Approximately 10^6 cells were taken from this suspension and stained for DNA with propidium iodide according to Feitz et al. (1985), and for CKs using the antibody RCK102 as described (Ramaekers et al. 1986).

Cell analysis was performed using a Cytofluorograph 50H (Ortho Instruments, Westwood, MA, USA). Propidium iodide was excited at 488 nm and fluorescence was measured using a 630 nm longpass filter. For determination of the DNA index chicken red blood cells served as an internal standard and human lymphocytes as an external standard (Jakobsen, 1983).

Results

Expression of cytokeratins, neurofilaments and vimentin

The expression patterns of CKs, NFs and vimentin in lung carcinoma cell lines as detected by the indirect immunofluorescence technique are shown in Table 2. The CK expression patterns are depicted in Fig. 1. Within the SCLC cell lines a subdivision can be made between the classic SCLC cell lines, which contain CKs but not vimentin or neurofilaments, transitional SCLC cell lines with a variable CK expression pattern and the expression of vimentin, and the variant SCLC cell lines, which in general do not contain CKs but contain vimentin and have a variable expression of neurofilaments. In all classic SCLC cell and in most transitional SCLC cell lines a positive staining reaction was observed with the broadly cross-reacting RCK102 antibody (CKs 5+8; Fig. 1A,B,C). Also in the large cell lung cancer (LCLC), the AC (Fig. 1D) and the SQC cell lines a strong staining reaction was seen with this antibody. Antibodies to stratified squamous cell epithelia, reacting with CK 4, 10 or 13, were reactive with two cell lines. One of these cell lines (GLC-3) was derived from a SCLC, which at the electron-microscopic level showed next to SCLC also AC differentiation, while the CK expression patterns indicate squamous cell differentiation. The other cell line (GLC-A2) was derived from an AC of the lung. The expression of CKs 4, 10 and 13 in this cell line clearly shows squamous cell differentiation, next to AC differentiation (a positive reaction with the CK 7 antibody). The classic SCLC cell lines reacted in general in most cells with the antibodies to CK 19 (Fig. 1E,G), while the transitional SCLC cell lines reacted only in part of the cells or not at all (Fig. 1F) with these antibodies. In non-SCLC cell lines a reaction pattern varying from only a weak reaction in some cells to a very strong reaction in all cells (Fig. 1H) was seen with the CK 19 antibodies. The monoclonal antibodies to CK 18 reacted with all classic (Fig. 1I,K), three out of four transitional (Fig. 1J) and three variant SCLC cell lines, and a strong reaction with all non-SCLC cell lines could be observed (Fig. 1L).

The antibody to CK 7 reacted with only one out of 19 SCLC cell lines in some of the tumour cells (Fig. 1M,N,O), while in contrast all but one of the non-SCLC cell lines were positive with this antibody (Fig. 1P).

Neurofilaments were present in seven out of ten variant SCLC cell lines. A filamentous staining reaction was not seen in the other types of SCLC cell lines with the NF antibodies. In only one out of nine non-SCLC cell lines was a reaction seen with the NF antibodies.

Vimentin was expressed in variable amounts, ranging from no reaction in the classic SCLC cell lines (see also Broers et al. 1985, 1986) to a strong filamentous staining reacting in adherent growing cells of most AC cell lines.

Our immunofluorescence findings on the CK expression in lung cancer cell lines were confirmed using the immunoblotting technique. Fig. 2 shows the results of one-dimensional immunoblotting assays of an AC cell line (A) and two classic SCLC cell lines (B). For comparison, the same nitrocellulose blots were subsequently incubated with different CK antibodies. Using LP2K, the presence of CK 19 could be demonstrated in the AC cell line (A, lane 1) and in one classic SCLC cell line (B, lane 2). CKs 8 and 18 were present in all three cell lines as detected by antibodies RCK102 and RCK106, respectively (A, lane 2; B, lanes 3, 4). Application of the CK 18 antibody RCK106 alone showed the presence of large amounts of CK 18 in the AC cell line (A, lane 3) as compared to a weak reaction in the two SCLC cell lines (B, lanes 5, 6). When applying the CK 7 antibody on these blots, an additional band was seen in the AC cell line (A, lane 4) but not in the SCLC cell lines (B, lanes 7, 8). Probably owing to breakdown of CK polypeptides, a phenomenon often occurring as a result of (limited) proteolysis by an endogenous protease (see also Schiller & Franke, 1983), additional bands can be seen, which are denoted by arrows. In immunoblotting

Fig. 1. Expression of cytokeratins (CKs) in classic small cell lung cancer (SCLC) cell line GLC1-M13 (A,E,I,M), transitional SCLC cell line SCLC-24H (B,F,J,N), classic SCLC cell line NCI-H449 (C,G,K,O), and in adenocarcinoma (AC) cell line NCI-H125 (D,H,L,P) as monitored by (chain-specific) monoclonal antibodies in the indirect immunofluorescence technique. A strong filamentous reaction pattern is seen with the broadly cross-reacting antibody RCK102 in all classic and most transitional SCLC cell lines (A, ×460; B, ×400; C, ×460), and in all AC cell lines (D, ×300). A variable reaction pattern is seen in SCLC cell lines using LP2K, specific for CK 19, showing a clearly filamentous staining reaction in the classic SCLC cell lines (E, ×360; G, ×410), while no reaction is seen in the transitional SCLC cell line (F, ×460). A strong reaction is seen with LP2K in the AC cell line (H, ×300). All CK positive SCLC cell lines contained CK 18 as demonstrated with RCK106 (I, ×460; J, ×410; K, ×460), while also the AC cell line showed a strong reaction with this antibody (L, ×300). In SCLC cell lines no reaction was seen with the CK 7 antibody RCK105 (M, ×460; N, ×410), except for cell line NCI-H449, in which part of the cells showed a positive reaction (O, ×460). Most cells of the AC cell line showed a strong staining reaction with RCK 105 (P, ×300).

studies of variant SCLC cell lines no CKs could be demonstrated (results not shown).

Coexpression of cytokeratins and neurofilaments

Cell line GLC-A1 was derived from an AC of the lung. In contrast to other AC cell lines (see Table 2), very few of its cells react with the CK 7 antibody, and many cells do not seem to contain any CKs at all. Fig. 3A shows that some cells of GLC-A1 react with RCK102, while others do not. In addition, an expression of NF proteins can be seen in some of the tumour cells (Fig. 3B). Quantification of labelled cells showed a reaction with RCK102 in about 7 % of all cells, and a reaction with the pNF160 antibody in about 30 %. Approximately 5 % of all cells showed a coexpression of CKs and NFs, i.e. reacting
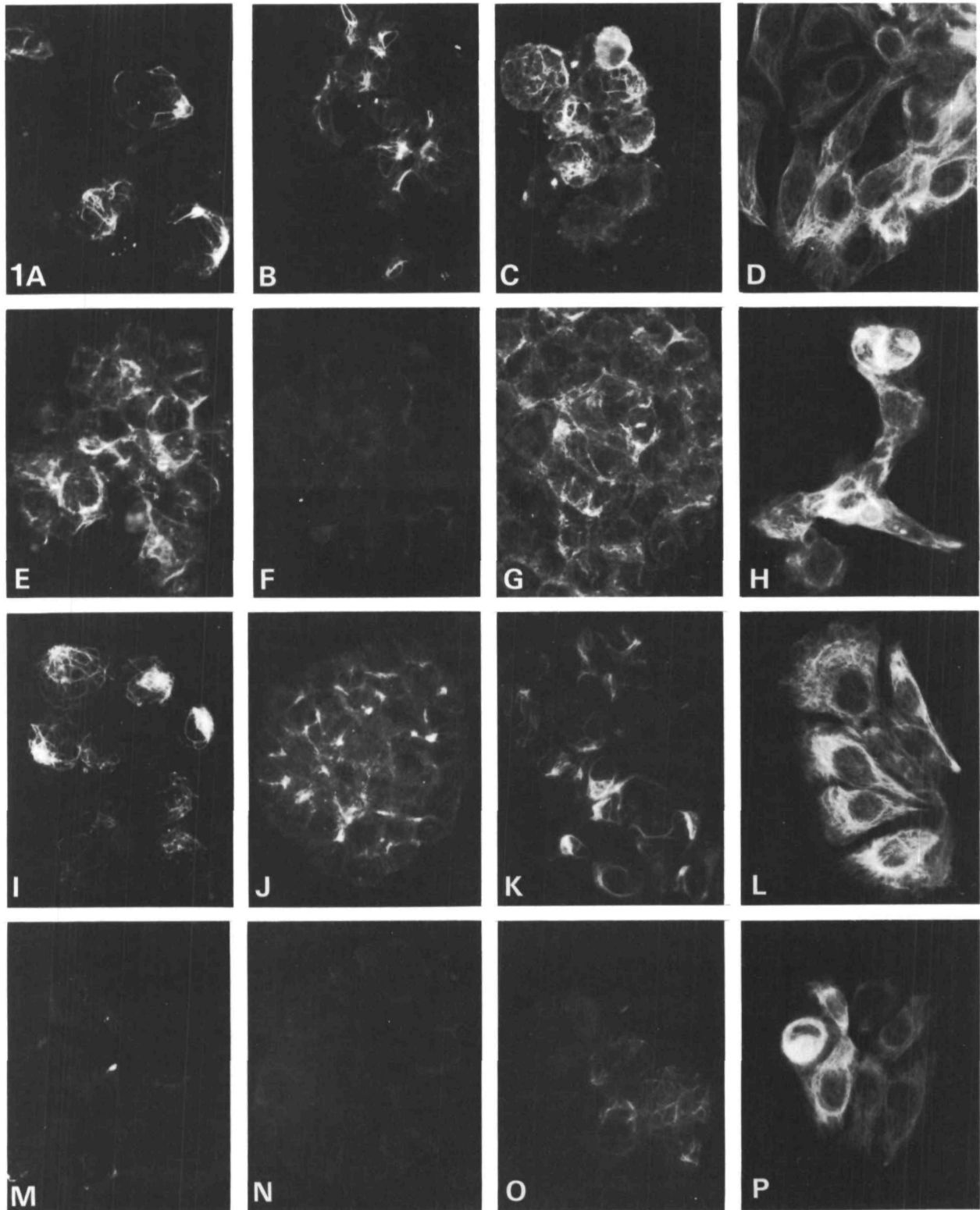
Table 2. *Reaction patterns of lung carcinoma cell lines with antibodies to intermediate filaments*

Cell line	RCK102 (5+8)*	RCK105 (7)	RGE53/ RCK106 (18)	LP2K/ BA17 (19)	6B10 (4)	1C7/2D7 (13)	RKSE60 (10)	MNF	pNF68	pNF160	pNF200	RV202	pVim
SCLC													
Classic													
NCI-H128	++	–	++	+	–	–	–	–	–	–	–	–	–
NCI-H449	+	+/–	+	++	–	–	–	–	–	–	–	–	–
NCI-H69	+	–	+/–	+	–	–	–	–	–	–	–	–	–
GLC1-M13	+	–	+/–	+/–	–	–	–	–	–	⊤	–	–	–
NL-SCLC2	++	–	++	+/–	–	–	–	–	–	–	–	–	–
Transitional													
SCLC-16HC	+/–	–	+/–	+/–	–	–	–	–	Dot-like	–	–	+/–	+/–
SCLC-22H	++	–	+	+	–	–	–	–	–	–	–	+/–	+/–
SCLC-24H	++	–	++	–	–	–	–	–	–	–	–	+/–	+/–
SCLC-86M1	–	–	–	–	–	–	–	–	–	–	–	+/–	+/–
Variant													
NCI-N417	–	–	–	–	–	–	–	–	–	–	–	+/–	+/–
NCI-H524	–	–	–	–	–	–	–	–	++	++	–	+/–	+/–
NCI-H82	–	–	–	–	–	–	–	++	++	++	++	+/–	+/–
GLC-1	–	–	–	–	–	–	–	–	–	–	–	+	+
GLC-2	–	–	–	–	–	–	–	+/–	++	++	–	+	+
GLC-3	+/–	–	+/–	+/–	Some c.	Some c.	Some c.	–	–	–	–	+/–	+/–
GLC-5	Some c.	–	Some c.	–	–	–	–	+/–	++	++	+/–	+	+
NL-SCLC3p10	–	–	–	–	–	–	–	+/–	+/–	+/–	+/–	+/–	+/–
SCLC-21H	+/–	–	+/–	–	–	–	–	–	Dot-like	–	–	+/–	+/–
SCLC-16HV	–	–	–	–	–	–	–	+/–	+/–	+/–	+/–	+/–	+/–
Large cell													
LCLC-103H	++	–	+/–	+/–	–	–	–	–	–	–	–	+	+
Squamous cell													
EPLC-32M1	++	+/–	++	+/–	–	–	–	–	–	–	–	+	+
EPLC-65M2	++	++	++	+/–	–	–	–	–	–	–	–	+	+
Adenocarcinoma													
NCI-H23	+/–	Some c.	+/–	–	–	–	–	–	–	–	–	++	++
NCI-H125	++	++	++	++	–	–	–	–	–	–	–	+	+
NL-AC1	++	++	++	++	–	–	–	–	–	–	–	++	++
GLC-A1	+/–	Some c.	+/–	+/–	–	–	–	+/–	+/–	+/–	+/–	+	+
GLC-A2	++	++	+/–	++	+/–	+/–	+/–	–	–	–	–	+	+
GLC-A3	++	++	++	++	–	–	–	–	–	–	–	+	+

++, strong reactivity in all cells; +, reactivity in more than 90% of all cells; +/–, reactivity in part of the cells (10–90%); Some c., reactivity in less than 10% of all cells; Dot-like, a dot-like reaction in a small part of all cells; –, no reaction in any cells.
* (5+8), CK polypeptide(s) recognized according to the catalogue of Moll *et al.* (1982).

with RCK102 and with pNF160 in the same cells. Double immunofluorescence staining showed that some cells clearly coexpress CKs and NFs (compare Fig. 3C and D, E and F), while other cells express only NFs and no CKs (Fig. 3C,D). Staining of cell nuclei in the immunolabelled preparations (Fig. 3G) showed that most cells in the GLC-A1 culture do not express IFPs of the CK or NF type. Fig. 4 shows the expression of CKs (red fluorescence) and NFs (green fluorescence) in different cells of the GLC-A1 culture, with cell nuclei stained blue. The micrograph clearly shows the coexistence of cells expressing different IF types in this culture.

GLC-5 was initiated from a lymph node metastasis of a SCLC expressing NFs next to CKs (de Leij *et al.* 1986). In early passages of this cell line only NFs were found, while in later passages a coexpression of CKs and NFs was seen again. Subcloning of GLC-5 did not result in a separation of CK or NF positive cells, but after some time each cloned population contained cells positive for NFs and few cells positive for CKs. Cells expressing CKs

had the morphological appearance of classic SCLC cells, i.e. growing in very tight clusters of floating cells, while most of the cells expressing NFs grew as loose clusters, which tended to attach to the surface of culturing flasks.

Transitions of IFP patterns within SCLC cell lines

Subcloning of the variant SCLC cell line GLC-1 resulted in cell line GLC1-M13, having biochemically classic SCLC properties (de Leij *et al.* 1985) and expressing CKs in nearly all tumour cells (Fig. 1). The variant SCLC cell line GLC-1, however, does not express any CKs (Broers *et al.* 1985; de Leij *et al.* 1985; Tables 2, 3).

One of the SCLC cell lines, i.e. SCLC-16H, was initially characterized as a classic SCLC cell line (Bepler *et al.* 1987b,c) and later reclassified as a transitional SCLC cell line (Bepler *et al.* 1988b). After about one year of culturing (approximately 65 passages) this cell line had lost its transitional SCLC morphology and expression of L-dopa decarboxylase activity (Bepler *et al.* 1987b). An early passage of this cell line (SCLC-16HCp39, Table 3),
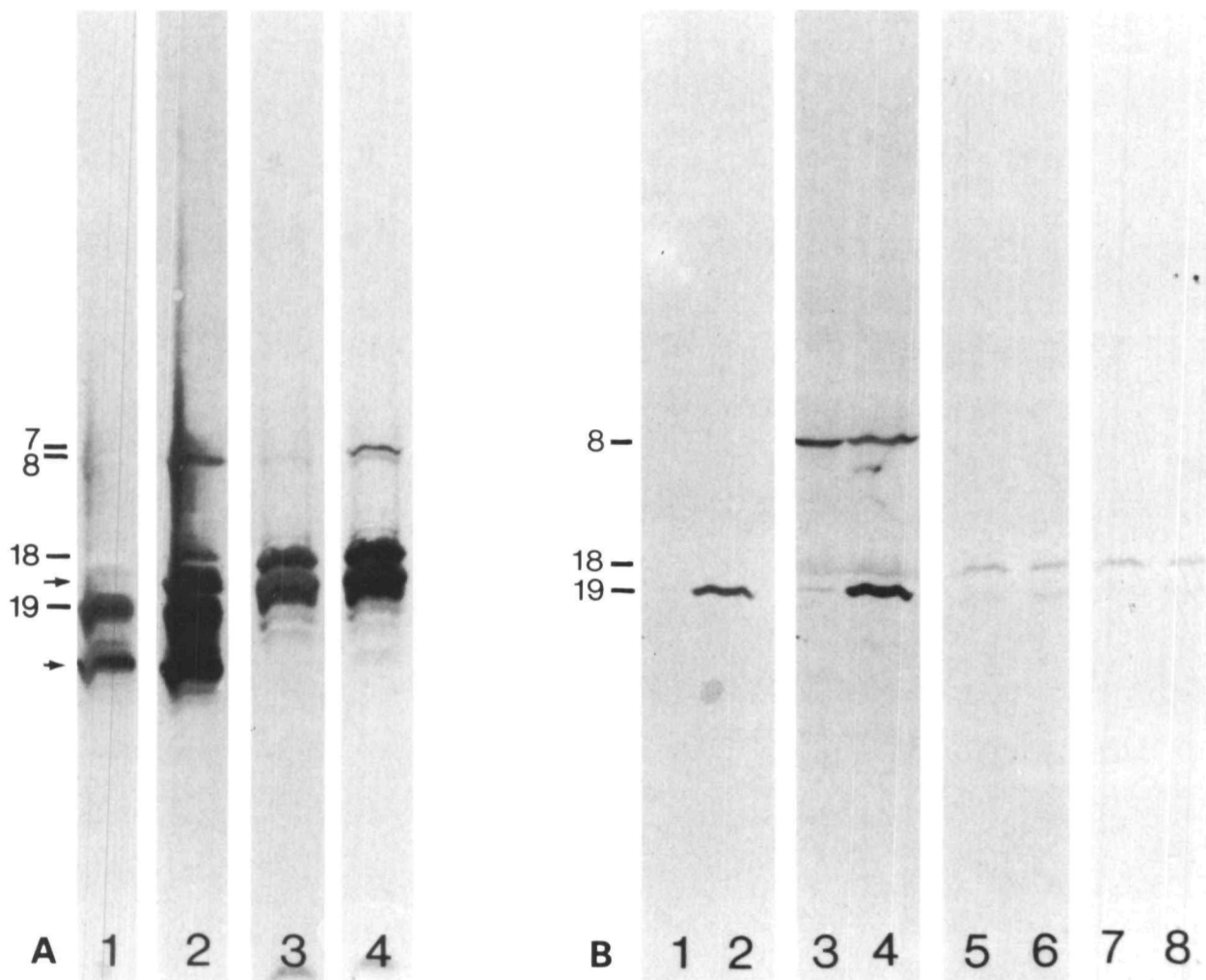
Fig. 2. One-dimensional immunoblotting assays using different chain-specific CK antibodies on cytoskeleton preparations from an AC cell line (NCI-H125; A), and from the two classic SCLC cell lines NL-SCLC2 (B, lanes 1, 3, 5, 7) and NCI-H69 (B, lanes 2, 4, 6, 8). On the basis of Coomassie Brilliant Blue staining patterns, comparable amounts of cytoskeletal proteins from each sample were applied. Immunoblotting with the antibody LP2K gave a band at the CK 19 level in the AC cell line (A, lane 1), and in the SCLC cell line NCI-H69 (B, lane 2). Subsequent incubation of the same immunoblots with a CK 18 antibody RCK106 and with RCK102, recognizing CKs 5 and 8, resulted in the appearance of bands at the level of CKs 18 and 8, respectively, in the AC cell line (A, lane 2), whereas in the SCLC cell lines a relatively strong band was detected in both cell lines at the CK 8 level. A much weaker reaction was seen at the CK 18 level (B, lanes 3 and 4). Incubation with RCK106 alone resulted in a strong reaction in the AC cell line (A, lane 3) and a weak, but evident reaction in the two SCLC cell lines at the CK 18 level (B, lanes 5 and 6). A subsequent incubation of these immunoblots with the antibody RCK105 gave an additional band at the CK 7 level in the AC cell line (A, lane 4), but not in the classic SCLC cell lines (B, lanes 7 and 8). Note the prominent bands in the AC cell line (denoted by arrows) representing breakdown products of CKs 18 and 19.

with typical transitional SCLC properties, showed the expression of CKs in most cells (Fig. 5A) and the absence of NFs (Fig. 5B). Some cells, however, showed a dot-like reaction pattern with the pNF68 antibody (Fig. 5C). A late passage of this cell line (SCLC-16HVp93), which was biochemically characterized as a variant SCLC cell line (Bepler et al. 1987b), did not express any CKs (Fig. 5D), but showed the presence of NFs in about 10% of the cells (Fig. 5E).

Cell line NCI-N417 did not seem to express CKs or NFs when introduced in our laboratory (Fig. 6A,B; see also Broers et al. 1985, 1986). However, when grown for more than 6 months in the same culture flask with regular changes of the culture medium, a subset of cells arose

(NCI-N417-A1) that grew firmly attached to the plastic. These cells appeared to express CKs (Fig. 6D,E), while some of the remaining floating cells of NCI-N417 showed a dot-like reaction with the CK 18 antibody RCK106 (Fig. 6C), but not with other CK antibodies. After several passages the formerly adhering cells were no longer attached, and the CK-positive subset of cells was lost. After another 6 months again some cells attached to the surface of the culture flasks (NCI-N417-A2). Both the floating and the attached cells now revealed a positive staining reaction with NF antibodies in about 10% of all cells (Fig. 6F,G).

A comparable switch in IFP expression was noticed in cell line NL-SCLC3. In frozen sections of the SCLC
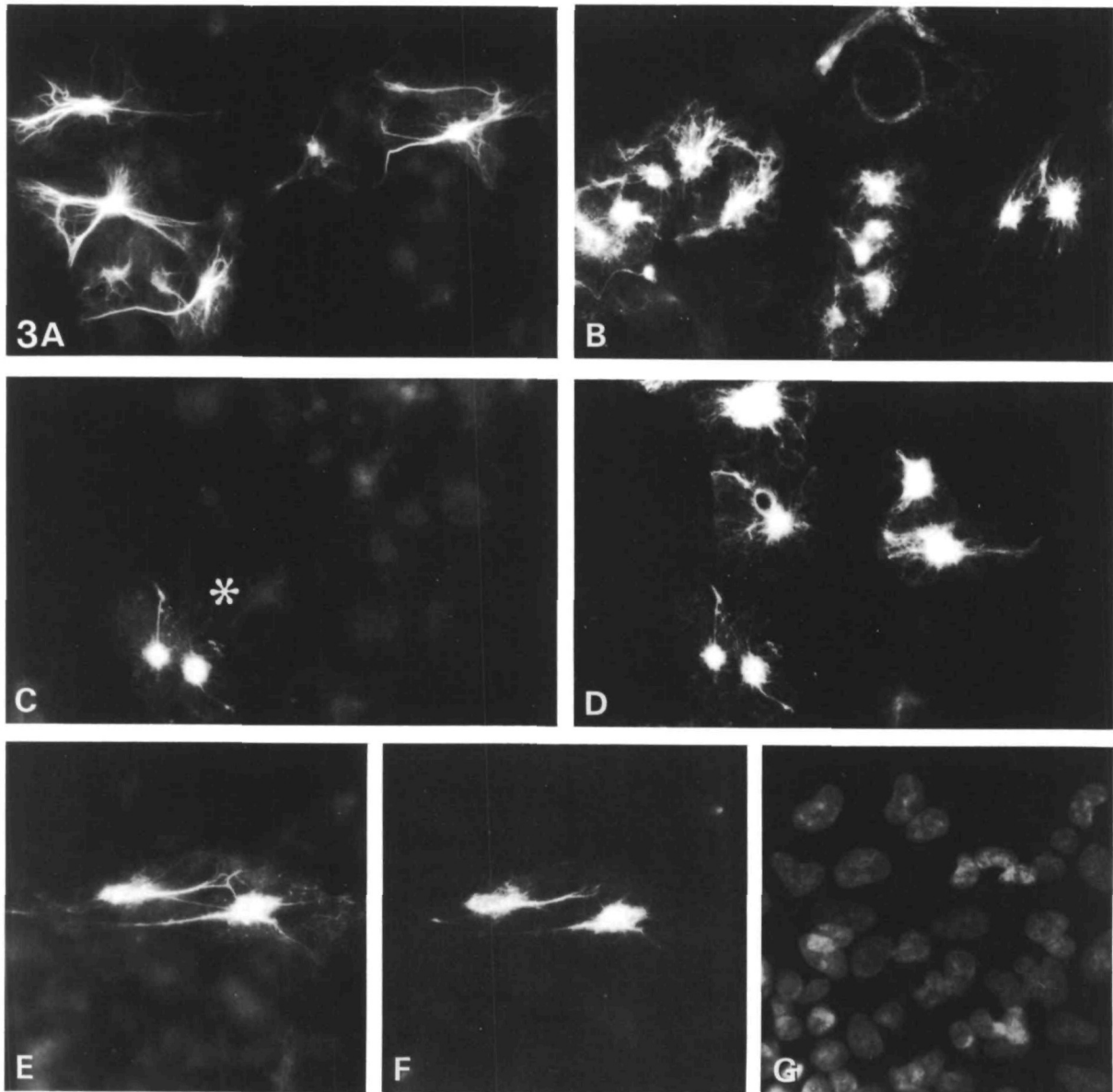
Fig. 3. Immunofluorescence staining patterns of cell line GLC-A1 derived from an AC of the lung. Part of the cells react strongly with RCK102 (A, ×385), while also a strong reactivity is seen with the pNF160 antibody (B, ×450). Double-label immunofluorescence studies (C–F) show that cells that are reactive with RCK102 (C, asterisk; ×450) are also stained by the pNF160 antibody (D, ×450). In addition, some cells that do not react with RCK102 are positive with the pNF160 antibody (D). Double-label immunofluorescence in combination with nuclear DNA staining with Hoechst (E,F,G) shows that also cells reactive with RCK106 (E, ×350), may react with the pNF160 antibody (F, ×350), while the distribution of fluorescence suggests colocalization of the two filament systems. The nuclear DNA staining (G, ×350), however, shows that most cells of this sample do not contain CKs of NFs.

from which this cell line was derived, no CKs or NFs could be detected in the tumour cells. The same holds true for the cell line up to passage 7. However, at passage 10 a small subset of cells was found that expressed NF proteins. With each subsequent passage the percentage of cells positive for NFs increased to about 50 % at passage 20.

Another type of change in IFP expression was seen in the variant SCLC cell lines NCI-H524 (Fig. 7) and NCI-H82 (Fig. 8). These cell lines normally grow as loosely packed floating aggregates, which do not attach to the

bottom of culturing flasks, and express NFs in all cells (Figs 7D, 8A). CKs can not be detected in these floating cells (Figs 7E, 8B). After a prolonged period of growing in the same culture flask, however, an increasing number of cells attached to the bottom (see, e.g., Fig. 7A,B,C). Immunofluorescence studies showed that these adhering cells not only lost their NF expression in part of the cells, but also that some of these cells initiated the (co-)expression of CKs (Figs 7F,G and 8C,D). Double immunofluoresence studies showed that in NCI-H524 some cells coexpress NFs and CKs, while other cells
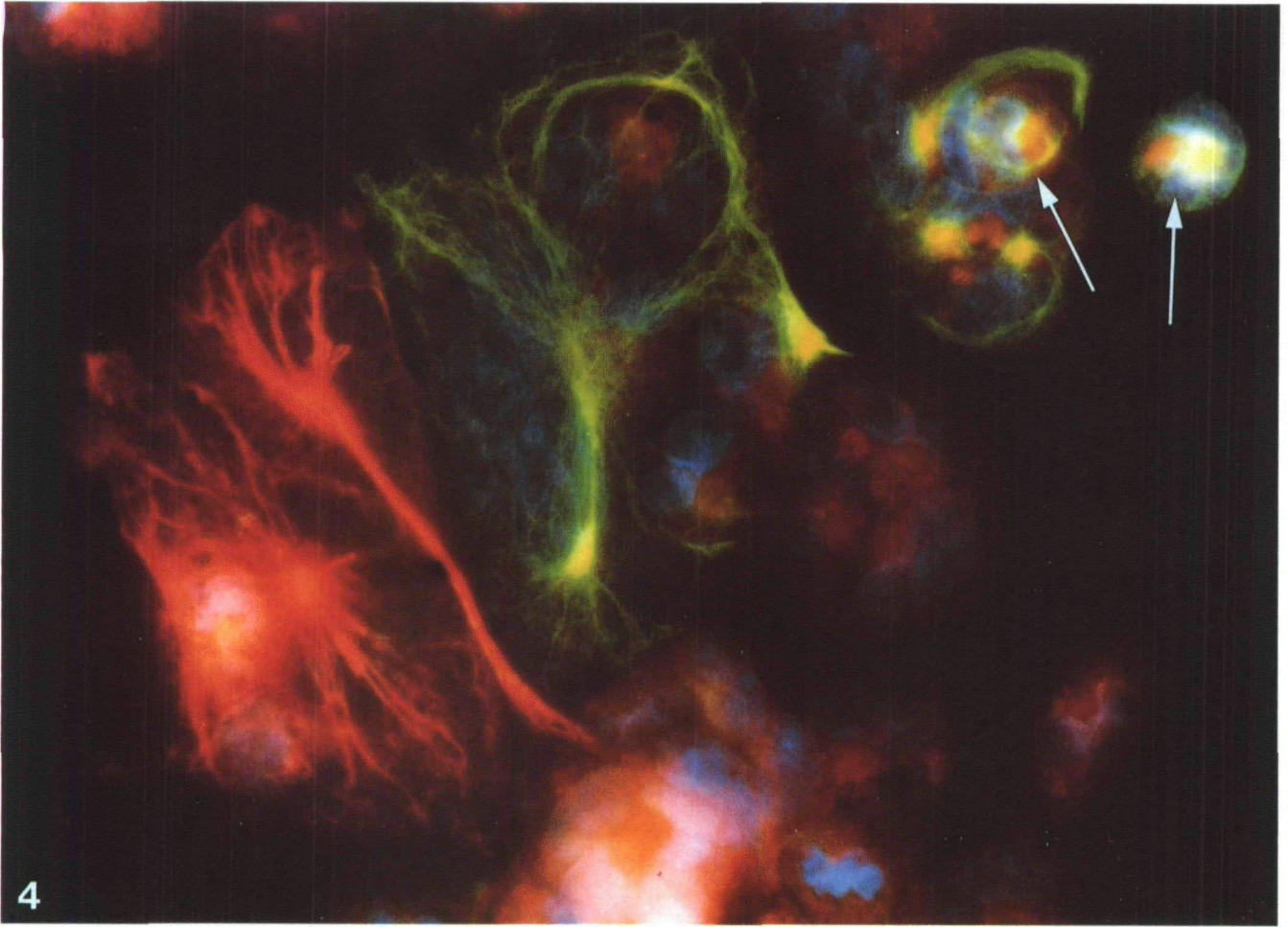
Fig. 4. Triple exposure of cell line GLC-A1 after incubation with RCK102 visualized with sheep anti-mouse Texas Red, pNF160 visualized with goat anti-rabbit FITC, and a nuclear DNA staining with Hoechst. Note the presence of cells containing only CKs (red fluorescence), the presence of cells containing only NFs (green fluorescence), and a possible coexpression of CKs and NFs in a third group of cells (yellow fluorescence, arrows); ×650.
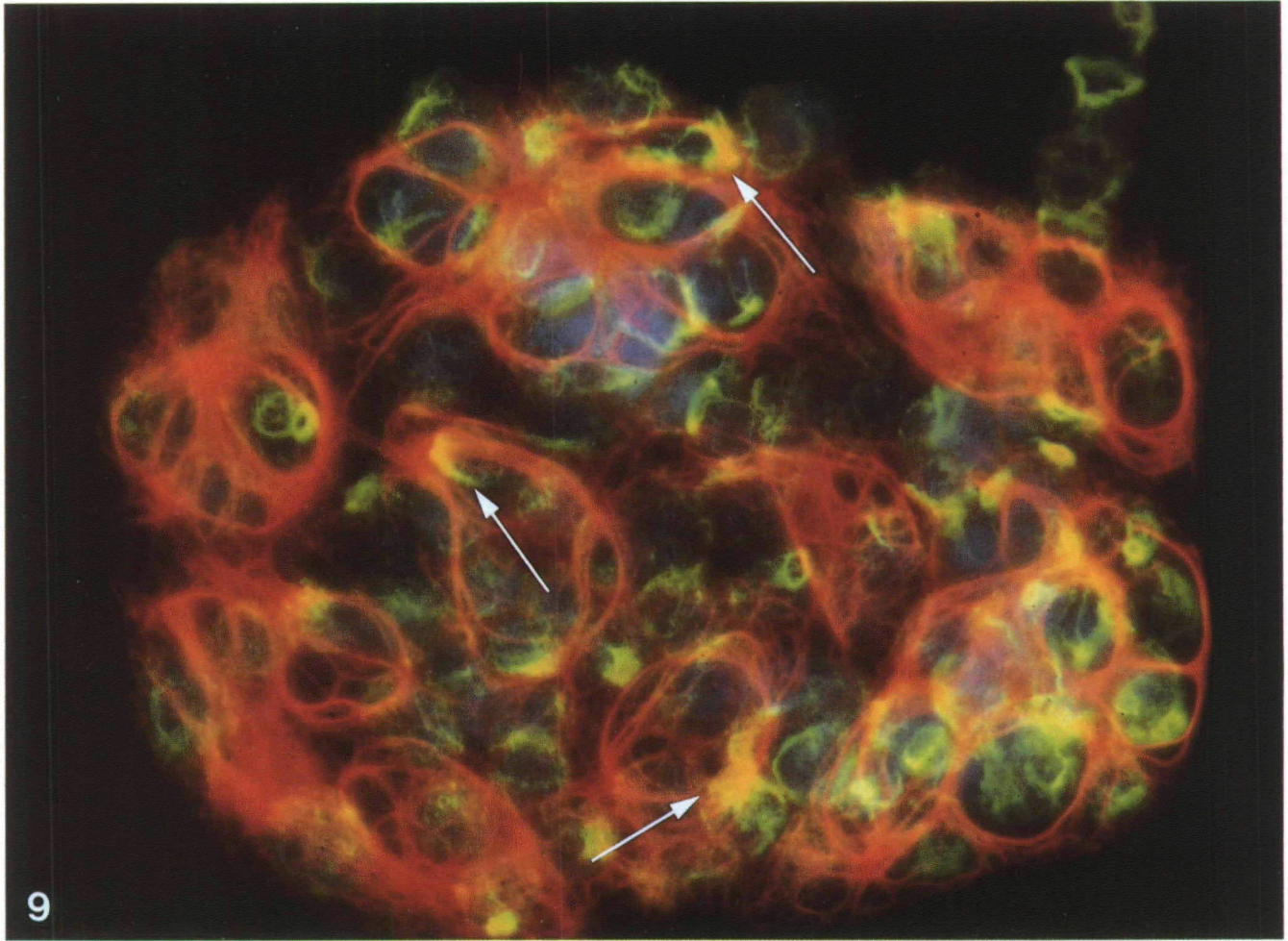
Fig. 9. Triple exposure of a double-label immunofluorescence experiment, with additional nuclear DNA staining of NCI-H82 (×650). Note the expression of CKs, reacting with RCK102 (red fluorescence) and the staining of NFs with the pNF160 antibody (green fluorescence). The yellow colour seen in some cells (arrows) is probably caused by the colocalization of CK and NF containing filaments.

Table 3. *Reaction pattern of cell lines exhibiting transitions in intermediate filament expression patterns*

Cell line	RCK102 (5+8)*	RCK105 (7)	RGE53/RCK106 (18)	LP2K/BA17 (19)	6B10 (4)	1C7/2D7 (13)	RKSE60 (10)	MNF	pNF68	pNF160	pNF200	RV202	pVim
GLC-1	−	−	−	−	−	−	−	−	−	−	−	+	+
GLC-1-M13	+	−	+/−	+/−	−	−	−	−	−	−	−	−	−
SCLC-16HC	+/−	−	+/−	+/−	−	−	−	−	Dot-like	−	−	+/−	+/−
SCLC-16HV	−	−	−	−	−	−	−	+/−	+/−	+/−	+/−	+/−	+/−
NCI-N417	−	−	Dot-like	−	−	−	−	−	−	−	−	+/−	+/−
NCI-N417-A1	++	−	++	++	−	−	−	−	−	−	−	++	++
NCI-N417-A2	−	−	−	−	−	−	−	+/−	+/−	+/−	+/−	+/−	+/−
NL-SCLC3p1	−	−	−	−	−	−	−	−	−	−	−	−	−
NL-SCLC3p10	−	−	−	−	−	−	−	+/−	+/−	+/−	+/−	+/−	+/−
NCI-H524	−	−	−	−	−	−	−	−	++	++	−	+/−	+/−
NCI-H524-A	+/−	−	+/−	−	−	−	−	−	+/−	+/−	−	+/−	+/−
NCI-H182	−	−	−	−	−	−	−	++	++	++	++	+/−	+/−
NCI-H182-A	++	−	++	−	−	−	−	+/−	+/−	+/−	+/−	+	+

++, strong reactivity in all cells; +, reactivity in more than 90% of all cells; +/−, reactivity in part of the cells (10–90%); Dot-like, a dot-like reaction in a small part of the cells; −, no reaction in any cells.
* (5+8), CK polypeptide(s) recognized according to the catalogue of Moll *et al.* (1982).
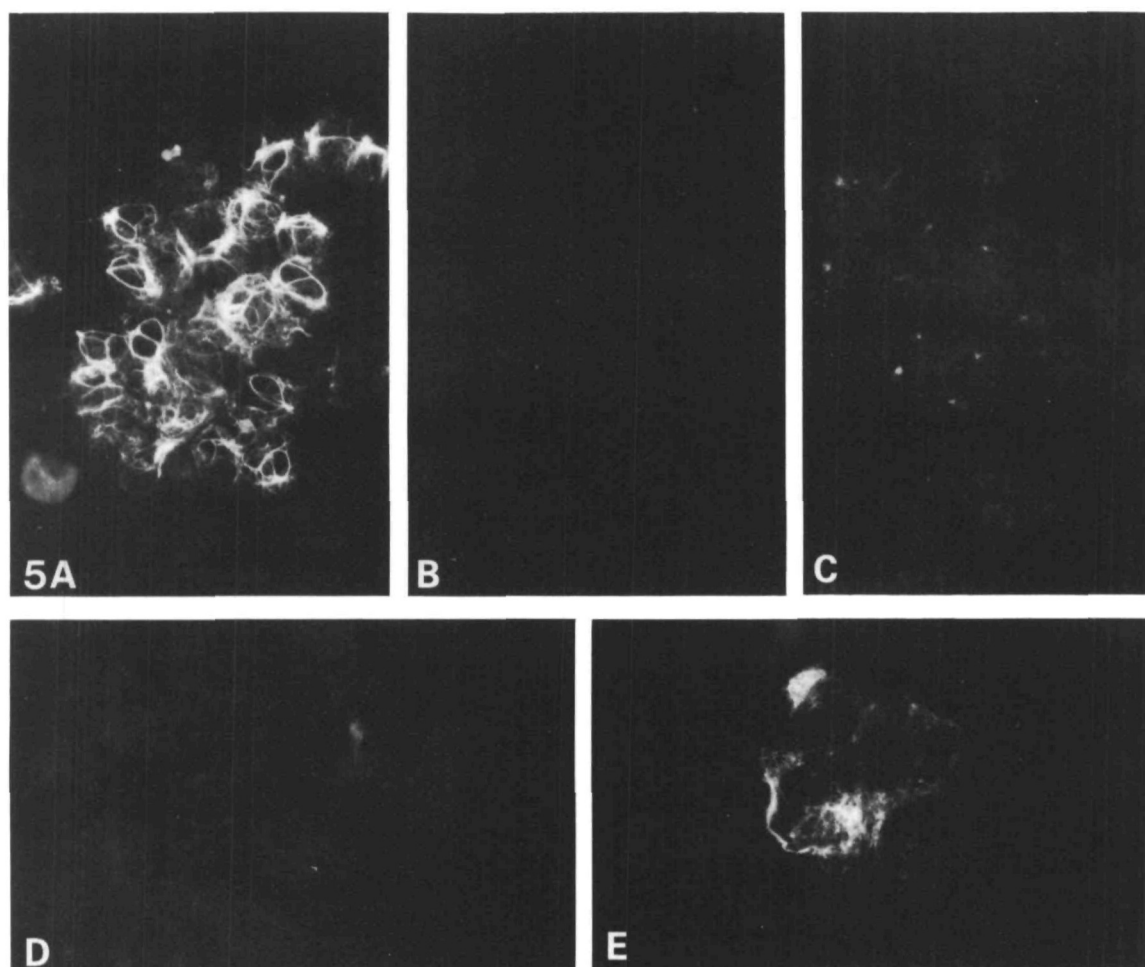

Fig. 5. Immunofluorescence staining patterns of SCLC-16H at an early passage number (p39; A,B,C) and at a late passage number (p93; D,E). During early passages most cells reacted strongly with the CK antibody RCK102 (A, ×450). Most cells did not react with the pNF68 antibody (B, ×475), while in some cells a dot-like reaction pattern with this antibody was seen (C, ×450). In the late passages all cells have lost CK expression (D, RCK102; ×450), while in about 10% of all cells a reaction with NF antibodies was found (E, pNF200; ×550).
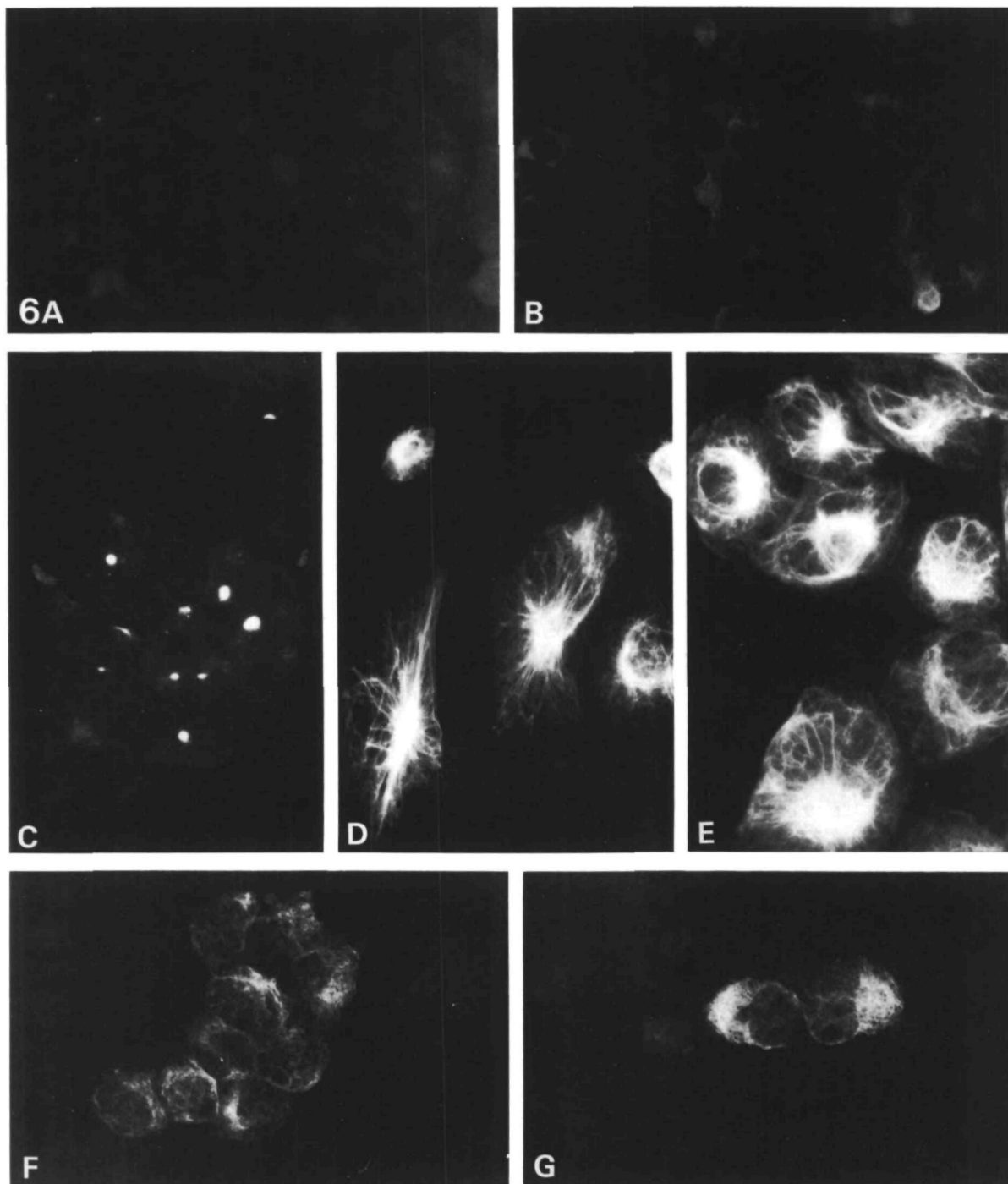
Fig. 6. Immunofluorescence staining patterns of NCI-N417 with (apparently) three different types of differentiation. Initially, this cell line did not express CKs, showing no reaction with RGE53 (A, ×360). Also no NFs were detectable with pNF200 (B, ×175). After prolonged culturing some cells started to attach to the culturing flask (C,D,E). At this stage some floating cells started to express CKs, a reaction with RCK106, in a dot-like manner (C, ×475). Cells that grew firmly adherent all contained CKs, reacting with RCK106 (D, ×410) and with RCK102 (E, ×435). Upon culture, CK positive cells were lost, while some cells started to express NFs, which were reactive with the pNF68 antibody (F, ×475) and with the pNF200 antibody (G, ×475).

express only NFs (cf. Fig. 7H,I). The same phenomenon can be observed in NCI-H82, where some cells express CKs (Fig. 8E,H) and other cells express NFs (Fig. 8F,I), while occasionally a clear coexpression of CKs and NFs can be observed (cf. Fig. 8H,asterisk, I).

Counterstaining of the nuclear DNA with Hoechst shows that most cells in NCI-H82 express either CKs or NFs (Fig. 8G,J). Coexpression of CKs and NFs is also clearly seen in Fig. 9, in which CKs are stained in red and NFs in green. The yellow colour may indicate colocalization of
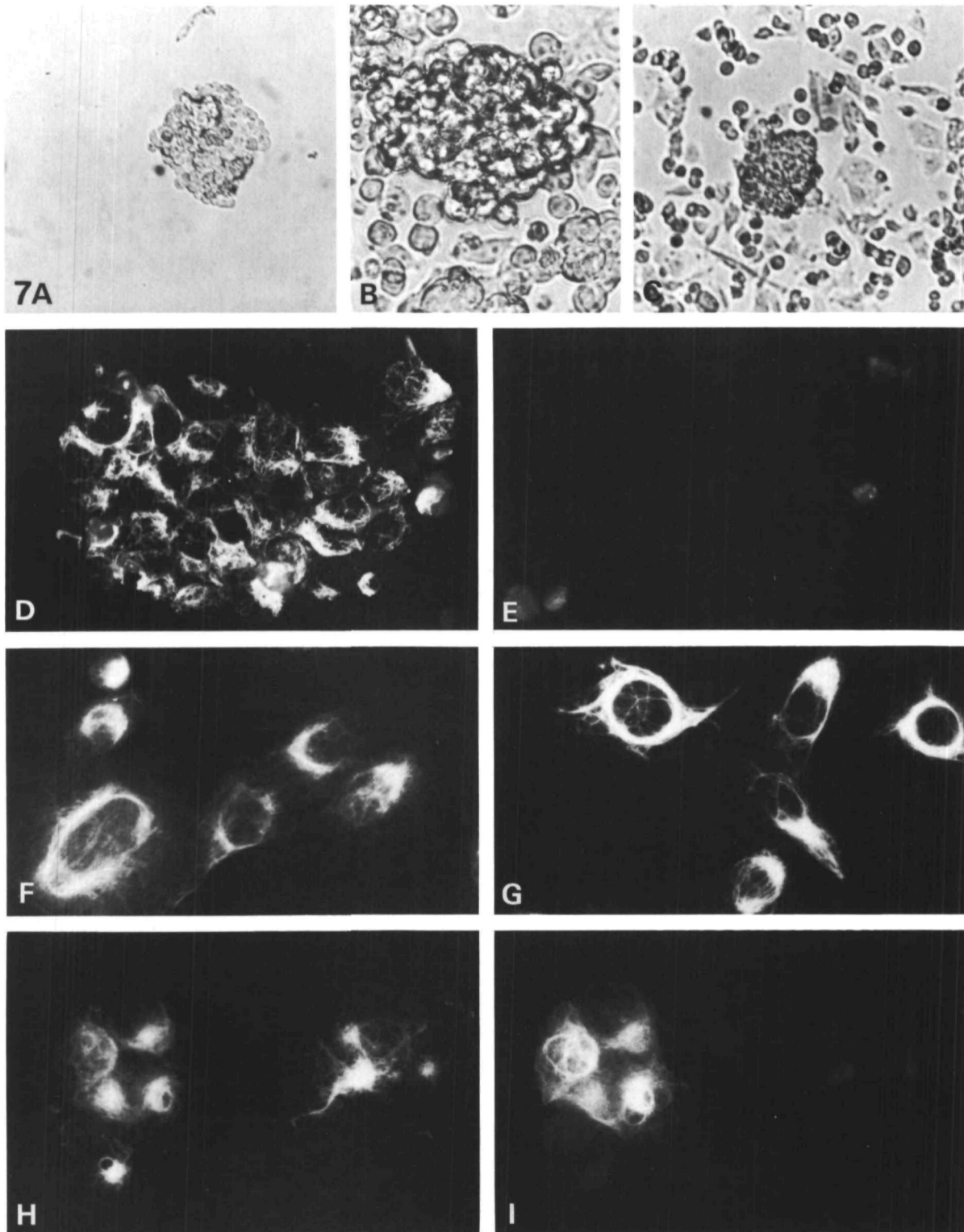
Fig. 7. Phase-contrast (A–C) and immunofluorescence (D–G) micrographs of the variant SCLC cell line NCI-H524. At first, this cell line grew as floating aggregates (A, ×105) with cells expressing NFs, showing a reaction in all cells with, for example, the pNF160 antibody (D, ×475). No cells could be detected to be positive for CKs (E, RCK106; ×360). Upon culturing, an increasing number of cells started to grow adherent (B, ×235; C, ×105). These adherent cells clearly expressed CKs, showing a reaction with RCK102 (F, ×435) and with RCK106 (G, ×435). Double-label immunofluorescence experiments (H,I) show that some cells that were reactive with the pNF160 antibody (H, ×475) also reacted with RCK102 (G, ×475).

CKs and NFs in certain cells.

Our immunofluorescence data were confirmed by immunoblotting experiments using RCK106 as a marker for CK expression and pNF68 as a NF marker (Fig. 10); A shows two weakly stained protein bands at the level of CK 18 (lane 1) and the somewhat stronger reaction with the $68 \times 10^3 M_r$ NF protein (lane 2) in GLC-A1, and in B

the early passage (p39) of SCLC-16HC was compared with a late passage (p93) of this cell line (SCLC-16HV). Lane 1 shows the presence of CK 18 in SCLC-16HC, while SCLC-16HV (lane 2) does not show a reaction with RCK106. Subsequent incubation with the pNF 68 antibody (lanes 3, 4) does not result in extra bands, probably due to the low number of cells expressing NFs in SCLC-
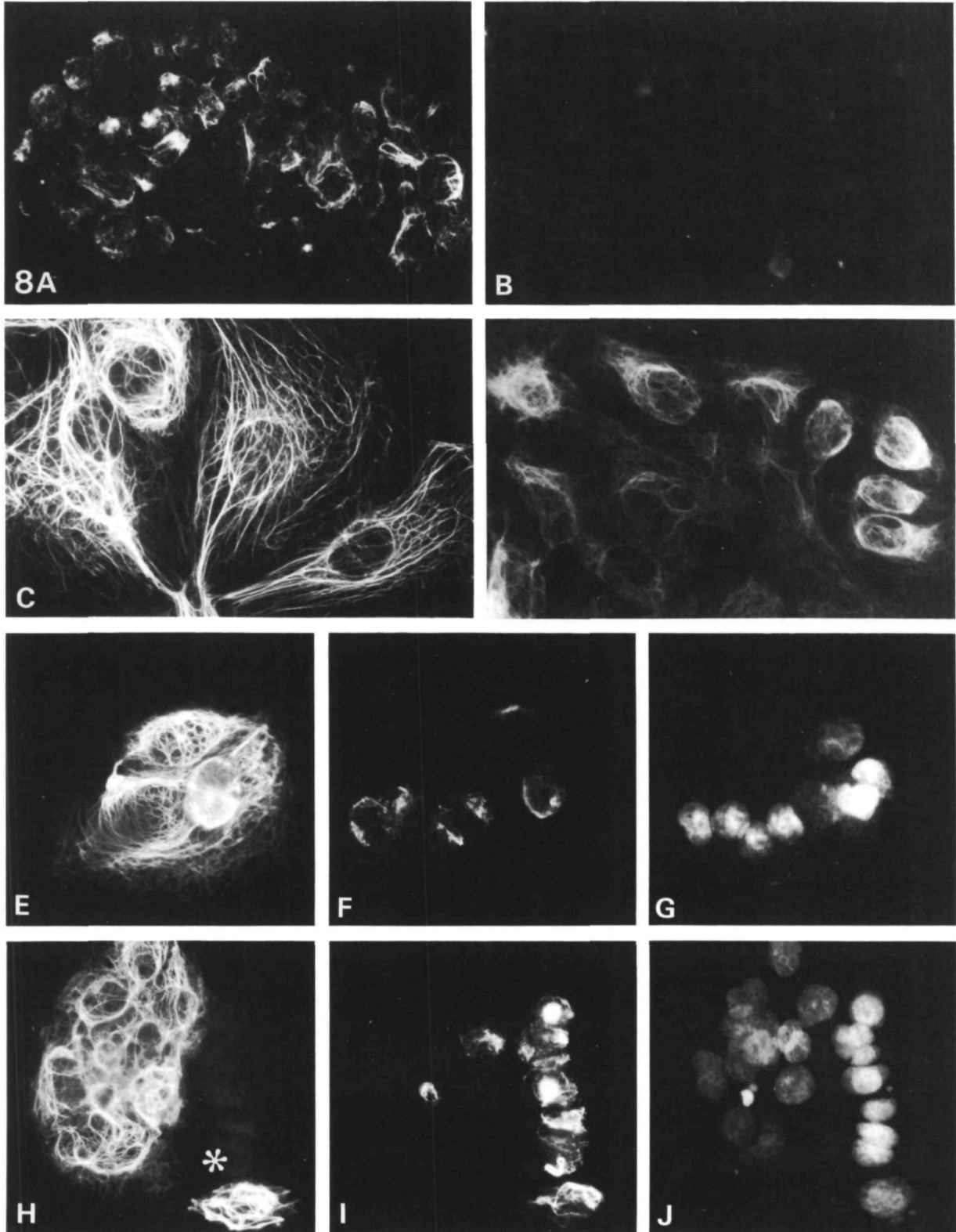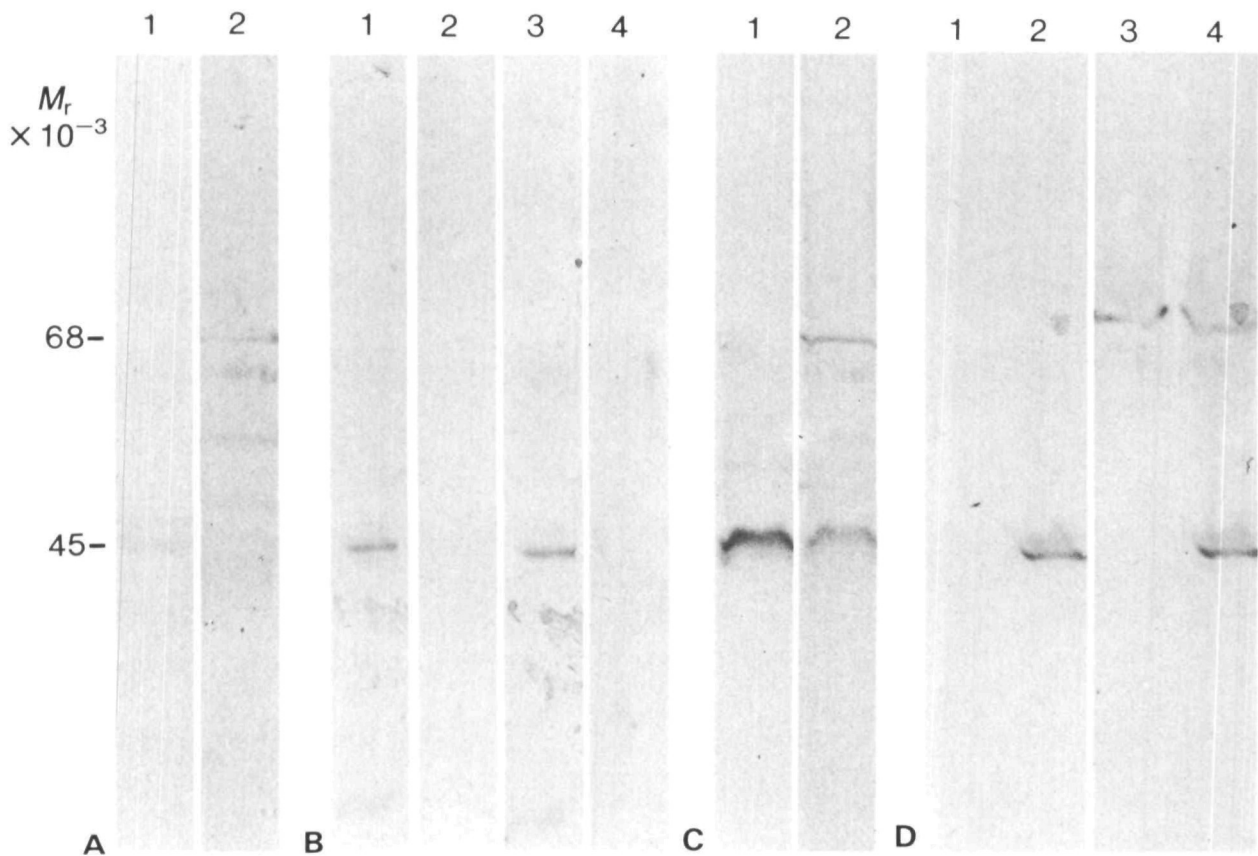
Fig. 10. One-dimensional immunoblotting of different lung cancer cell lines. A. Cell line GLC-A1 shows a faint (double) band at the $45\times10^3 M_r$ level with the CK 18 antibody RCK106 (lane 1). This weak reaction can be explained by the fact that CKs were detected in only 7% of all cells in immunofluorescence experiments. After subsequent incubation of the same immunoblot with the pNF68 antibody a stronger reaction is seen at the $68\times10^3 M_r$ level (lane 2). B. Early passage of SCLC-16HC (passage 39; lanes 1, 3) compared with a late passage (SCLC-16HV, passage 93; lanes 2, 4) of this cell line. SCLC-16HC shows a clear reaction with RCK106 (lane 1), while no reaction is seen with RCK106 in SCLC-16HV (lane 2). Incubation of the same blot with pNF68 did not result in an additional band at the $68\times10^3 M_r$ level in either cell line, confirming the immunofluorescence data of SCLC-16HC with this antibody. A discrepancy is seen with the positive immunofluorescence reaction with pNF68 in SCLC-16HV, which might be explained by the low number of cells reacting with this antibody. C. Adherent growing culture of the variant SCLC cell line NCI-H524, incubated with RCK106 (lane 1) and subsequently incubated with pNF68 (lane 2). Note the presence of the CK 18 band and the $68\times10^3 M_r$ NF band in this cell line. D. Comparison of the floating (lanes 1, 3) and the adherent growing cells (lanes 2, 4) of the variant SCLC cell line H82. Incubation with RCK106 results in a reaction at the $45\times10^3 M_r$ level with the adherent growing cells only (compare lanes 1, 2), while subsequent incubation with pNF68 shows that both cell types contain the $68\times10^3 M_r$ NF polypeptide.

Fig. 8. Immunofluorescence staining patterns of the variant SCLC cell line NCI-H82. This cell line, growing as floating aggregates, expressed NFs (A, pNF160; ×310) and did not express CKs (B, RCK106; ×420). However, after prolonged culturing some cells started to grow adherent, and selection of these cells resulted in a cell culture that was clearly positive with different CK antibodies, such as RCK102 (C, ×460) and RCK106 (D, ×410). Double-label immunofluorescence studies (compare E,F,G, with H,I,J) showed that within this cell culture some cells expressed CKs, reactive with RCK102 (E, ×410; H, ×345), while other cells were reactive with the pNF200 antibody (F, ×410) or with the pNF68 antibody (I, ×345). Nuclear DNA staining (G, ×410; J, ×345) showed that most cells were reactive with either CK or NF antibodies, while in one cell probably a coexpression of CKs and NFs was seen (compare H,I,J; asterisk).

16HV. Fig. 10C shows the presence of CK 18 (lane 1) and of the $68\times10^3 M_r$ NF protein (lane 2) in the subset of cells from NCI-H524 that grows as an attached monolayer. Fig. 10D shows the differences between CK and NF expression in the floating and the attached sublines of NCI-H82. Incubation with the CK 18 antibody shows the presence of this protein in the adherent cells (lane 2) and no reaction in the floating cells (lane 1). Subsequent incubation with the NF antibody shows that both cell types express the $68\times10^3 M_r$ NF protein (lanes 3, 4).

Monitoring of cross-contamination

To detect whether changes within cell lines might be due to possible cross-contamination with other cell lines simultaneously growing in our laboratory, flow cytometric analyses were performed at regular intervals. As an example, Fig. 11 shows the flow cytometric analysis of
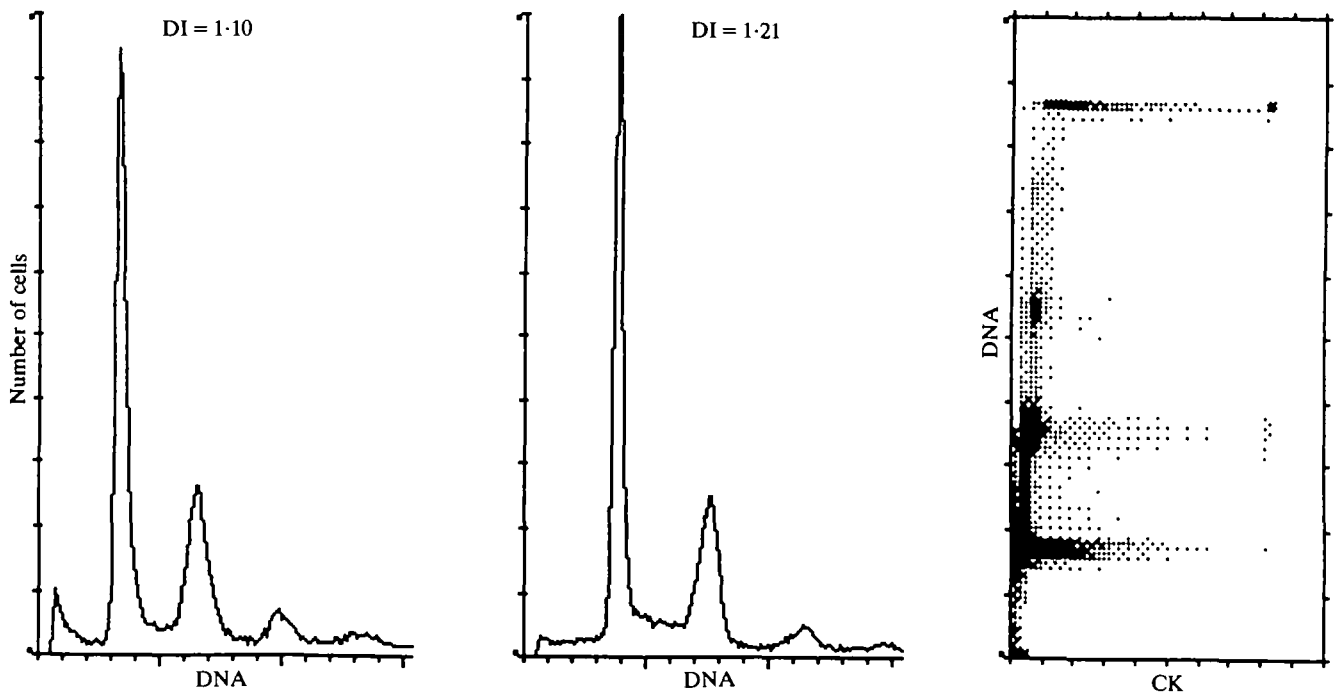
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Fig. 11. Comparison of flow cytometric analyses of floating (A) and adherent growing (B,C) cells of the variant SCLC cell line NCI-H524. The DNA indices (DI) found for both cell types did not differ significantly. C. Two-parameter flow cytometric analysis of cells from B separated according to their cytokeratin (CK) content, as determined with RCK102 (labelled with rabbit anti-mouse FITC), and DNA content as determined by labelling with propidium iodide. It was calculated that about 15 % of the adherent cells of NCI-H524 were positive with RCK102.

the floating fraction of NCI-H524, as compared with the adherent growing fraction of this cell line. No significant shift in DNA index (DI), nor extra DNA peaks, indicating a possible contaminating cell line, were observed (cf. Fig. 11A,B). The DI value of both cell lines varied between 1·10 and 1·23. In addition, the attached fraction was labelled with the CK antibody RCK102 and analysed in a two-dimensional fashion, in which DNA was stained with propidium iodide. The results show that about 15 % of the adherent cells contained CKs (Fig. 11C). Floating cells did not contain CKs (not shown).

The DI values of the two adherent growing variant SCLC cell lines (NCI-H82, DI = 1·20–1·22; NCI-H524, DI = 1·10–1·23) were compared with those for other adherent growing lung cancer cell lines such as EPLC-32M1 (DI = 1·50), EPLC-65M2 (DI = 1·55), LCLC-103 (DI = 3·27), NCI-H23 (DI = 1·60), NL-AC1 (DI = 1·52), and non-lung cancer epithelial cell lines growing in our laboratory, such as T24 (DI = 1·57) and HeLa (DI = 1·48). Since the DI values for the G_0/G_1 cells of these cell lines are all significantly higher than 1·23, a cross-contamination with these cell lines can be excluded. In addition, the CK and NF expression patterns of the adherent growing variant SCLC cell lines appeared to be unique for these two cell lines.

Discussion

In this paper we describe changes in IFP expression in lung cancer cell lines during long-term culture. We have

especially focused on CKs and NFs, since it is known that classic and variant SCLC cell lines exhibit different expression patterns of these IFPs (Broers et al. 1985, 1986).

In the last decade lung carcinoma cell lines that have retained many characteristics of the in vivo cancers have been established (Gazdar et al. 1981, 1985; Bergh et al. 1984; Carney et al. 1984, 1985a,b; Bepler et al. 1987b,c, 1988b). Most of these cell lines form tumours when injected into athymic nude mice with a histology similar to that of the original malignancy. Banks-Schlegel et al. (1985) stated that cell lines derived from different types of lung tumours continue to maintain a program of CK expression reflective of the native tumour.

Cytokeratins and neurofilaments in lung cancer cell lines

Our results on the IFP expression patterns in lung cancer cell lines are partly in accord with the findings in solid lung carcinomas with homogeneous morphology (Broers et al. 1988). In general, one can state that classic and transitional SCLCs and ACs contain CKs typical of simple epithelia, i.e. CKs 8, 18 and sometimes 19. In addition to these CKs, AC cell lines are characterized by the presence of CK 7, which is absent in SCLC. Variant SCLC cell lines in general do not contain CKs but may express NFs (see also Broers et al. 1985, 1986). Some exceptions, however, were seen.

(1) Two cell lines derived from SQCs (EPLC-32M1 and EPLC-65M2) did not express the typical SQC CKs, i.e. CKs 10 and 13, but contained CKs 7, 8 and 18,

occurring in simple epithelia, in this way resembling poorly differentiated SQC in their expression pattern (see also Broers *et al*. 1988). These changes are in accord with histological findings in mouse xenografts, in which these tumours were classified as undifferentiated large cell carcinomas (Bepler *et al*. 1988*b*). Furthermore, Blobel *et al*. (1984) have already shown that other cell lines derived from SQC mainly express CKs 7, 8 and 18.

(2) On the other hand, a cell line derived from an AC (GLC-A2) did express CKs 10 and 13, characteristic of SQC as well as CKs 7, 8 and 18, characteristic of ACs.

(3) Another cell line derived from an AC (GLC-A1) expressed NFs next to CKs, while CK 7, characteristic of ACs of the lung (see also Broers *et al*. 1988), was present in only a few cells of this culture. Therefore, on the basis of IFP expression patterns, this cell line resembles variant SCLC cell lines rather than AC cell lines.

(4) One of the SCLC cell lines (GLC-3) exhibited SQC differentiation as concluded from its IFP expression pattern. Interestingly, this cell line was previously shown to express AC differentiation characteristics at the electron microscopic level (de Leij *et al*. 1985).

These data clearly demonstrate heterogeneity within some cell lines in both degree and type of differentiation. However, results from most other cell lines show that cultured SCLC lines may serve as suitable models with which to study the biology of lung carcinoma subtypes (see also Gazdar & Minna, 1986).

Transitions in IFP expression patterns

In earlier studies, changes in growth pattern were observed during prolonged culturing of lung cancer cell lines; for instance, progression of classic to variant SCLC cell lines has been reported by several authors (Gazdar *et al*. 1981, 1985; Carney *et al*. 1985*b*; Graziano *et al*. 1987; Bepler *et al*. 1987*a,b*). Since classic SCLCs express CKs, while variant SCLCs may express NFs (Broers *et al*. 1985, 1986), we wondered whether such a progression would be accompanied by a change or loss of IFPs. In one of our cell lines (SCLC-16H), showing a progression towards variant SCLC, a transition from CK to NF expression was indeed seen.

Two cell lines (NL-SCLC3 and NCI-N417), which at first did not express CKs or NFs, initiated the expression of NFs upon prolonged culturing, suggesting that the absence of CKs and NFs in some SCLC cell lines as well as in some solid SCLC tumour parts (see also Broers *et al*. 1987) may be a temporary event occurring in some SCLC with a type of differentiation between classic and variant, the so-called transitional type of SCLC (Bepler *et al*. 1987*a*). *In vitro* the transitional cell type, in contrast to the classic cell type, expresses vimentin. Cytokeratin expression is found in varying degrees, and neurofilaments are absent or barely demonstrable. It remains to be determined to what extent the transitional type of SCLC can be compared with the so-called biochemical variant type of SCLC as defined by Gazdar *et al*. (1985). Examination of IFP expression patterns in these biochemical variant SCLC cell lines might elucidate this issue.

Another conversion that seems to occur within our cell lines is that from variant SCLC to non-SCLC differentiation, as described by Gazdar *et al*. (1981). *In vitro* non-SCLC cell lines, which do not express the neuroendocrine-related biomarkers present in SCLC (Carney *et al*. 1985*a,b*; Gazdar *et al*. 1985), grow as monolayers, firmly attached to the surface of culturing flasks. By selecting cells within the variant SCLC cell lines that tended to attach, we were able to grow sublines consisting of predominantly attached cells. Interestingly, these cells lose the expression of the neuroendocrine marker MOC-1 (results not shown), and start to express CKs next to NFs, resulting in a coexpression of CKs and NFs within these cell lines or within individual cells. Whether these cells lose the expression of other biomarkers, characteristic of (variant) SCLC, remains to be examined.

As far as the implications of these findings can be extrapolated to patient tumours it is important to note that there is growing evidence of heterogeneity of lung carcinomas. This heterogeneity can apparently also be found in (some) lung cancer cell lines and, even more interestingly, in cloned cell lines. Progression from classic to variant SCLC has been reported *in vivo* (Abeloff *et al*. 1979), especially after chemotherapy and/or irradiation of patients. Also conversions from SCLC to non-SCLC (Abeloff *et al*. 1979; Sehested *et al*. 1986) are often seen. In general, we can state that conversions from one cell type to another within cell lines are accompanied by a change in IFP expression patterns. The conversions observed in the cell lines closely resemble those occurring *in vivo*. Therefore, our *in vitro* findings strongly support the hypothesis that different types of lung carcinomas may have a common origin (see also Gazdar *et al*. 1981; McDowell & Trump, 1983) and as a result heterogeneity in lung tumour types as well as conversions from one tumour type to another are likely to occur as a result of (trans)differentiation rather than derivation (Gould, 1986).

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