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Research Article

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An Adherent Subline of a Unique Small-Cell Lung Cancer Cell Line Downregulates Antigens of the Neural Cell Adhesion Molecule

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Abstract

Small-cell lung cancer (SCLC) lines are distinguished from non–small-cell lung cancer (NSCLC) lines by their growth in floating aggregates, in contrast to the adherent monolayers formed by NSCLC cells in culture. Of 50 well-characterized SCLC lines recently described by the National Cancer Institute (NCI)-Navy Medical Oncology Branch, only four variant cell lines (SCLC-v) grew as adherent monolayers. One line, NCI-H446, was unique in growing long-term with coexisting floating and surface adherent subpopulations. We have physically segregated these two populations over many passages in vitro to enrich for relatively pure cultures of floating and adherent cells. No differences in c-*myc* expression, keratin pattern, or cytogenetic appearance were found between the adherent and floating sublines. However, expression of the neuroendocrine marker neuron-specific enolase in the floating cells was three times that found in the adherent cells. The floating subline also had much greater surface expression of neuroendocrine tumor antigens detected by monoclonal antibodies UJ13A and HNK-1, which have been recently shown to detect the neural cell adhesion molecule (NCAM) on SCLC cells. Two other adherent SCLC-v lines were also found to be unreactive with UJ13A and HNK-1, generalizing the association between NCAM expression and the growth of most SCLC cultures as floating aggregates. In conclusion, we have an interesting model to study expression of NCAM as related to the adhesive properties of SCLC cells. (*J. Clin. Invest.* 1990. 86:1848–1854.) Key words: lung cancer • neural cell adhesion molecule • neuron-specific enolase

Introduction

A striking difference between small-cell lung cancer (SCLC)[1] and non–small-cell lung cancer (NSCLC) cells in vitro is that SCLC cells generally float in spheroid aggregates and NSCLC cells grow as adherent monolayers (1). Classic SCLC cells tend to grow in tight spheroids with central necrosis whereas variant SCLC cells (SCLC-v) grow in looser branching chains (2). A small number of SCLC-v lines have been noted to grow in adherent monolayers in vitro (3). These adherent variant cells, referred to as having type 4 morphology, are clearly SCLC by other morphologic, cytogenetic, and biochemical features. In the experience of the National Cancer Institute (NCI)-Navy Medical Oncology Branch, only one well-characterized line has been described as growing with coexisting adherent and floating subpopulations, although similar lines have been described in the literature (3). This line, NCI-H446, was derived from the pleural effusion of a SCLC patient and expresses the SCLC biochemical markers neuron-specific enolase (NSE) and the brain isoenzyme of creatine kinase (4, 5). NCI-H446 cells form tumors with SCLC-v morphology (3), when implanted in nude mice.

The adherent NCI-H446 cells, although distinct from NSCLC cells by electron microscopy, mimic the culture properties of NSCLC (3). We were interested in whether the adherent SCLC cells had lost any of the neuroendocrine or immunologic markers distinguishing SCLC from NSCLC. By developing floating and surface adherent sublines of the NCI-H446 line, we were able to directly examine the association of growth as an adherent monolayer on several characteristic amine precursor uptake and decarboxylation or neuroendocrine properties of SCLC cells.

Moolenaar et al. (6) and Kibbelaar et al. (7) have recently demonstrated that a dominant surface antigen of SCLC cells, recognized by numerous monoclonal antibodies (MAbs) and termed SCLC cluster 1 (SC-1), is actually a neural cell adhesion molecule (NCAM)-related sialoglycoprotein. We have been able to use our adherent and floating sublines of H446 to examine expression of NCAM determinants as related to the adherent properties of SCLC cells.

Methods

Cell cultures. We used the previously described human SCLC-v cell line NCI-H446, derived from the pleural effusion of a patient who had relapsed with disease after treatment with combination chemotherapy (3). Like most SCLC-v lines, H446 cells do not express L-dopa decarboxylase or bombesin-like immunoreactivity (8). The classic SCLC lines H209 and H146, the SCLC-v line N417 and the NSCLC lines H157, H125, and H522 were used for comparative studies and have been previously described (9). Two unusual SCLC lines, H1607 and H196-B, which form adherent monolayers in vitro, were also used. These two cell lines, characterized as having type 4 SCLC morphology, were derived from patients with SCLC histology (3). H196-B has been further characterized, and forms typical SCLC tumors of an intermediate type when inoculated into nude mice, as well as having detectable SCLC markers of NSE and creatine kinase (3). All cell lines were tested and found to be free of *Mycoplasma* contamination.

1. *Abbreviations used in this paper:* NCAM, neural cell adhesion molecule; NCI, National Cancer Institute; NSCLC, non–small-cell lung cancer; NSE, neuron-specific enolase; SCLC and SCLC-v, small-cell lung cancer and variant SCLC; SC-1, SCLC cluster 1.

Culture conditions and experimental protocols were as previously described (3). NSCLC cells were maintained in RPMI-1640 in the presence of 10% fetal bovine serum and 2 mM L-glutamine. The SCLC cells were grown in 2% fetal bovine serum with the addition of hydrocortisone, insulin, transferrin, estradiol, and selenium reagents as previously described (10). RPMI-1640 medium and glutamine were from Grand Island Biological Co., Grand Island, NY; the serum from Hyclone Laboratories, Logan, UT; the insulin, transferrin, and sodium selenite from Collaborative Research Inc., Lexington, MA; and the 17-B-estradiol and hydrocortisone from Sigma Chemical Co., St. Louis, MO.

Creation of floating and adherent subpopulations of NCI-H446. Stable populations of floating H446 cells were created by serially removing the nonadherent cells to new flasks. Loosely adherent cells were then removed from the original flask by trituration and the most adherent cells were kept. Similar measures over some 40 passages created relatively pure populations of floating and sticking cells which were stable for months in culture. Subcloning of floating and adherent cells used for subsequent assays was performed by selecting single colonies from clonogenic assays.

Calculation of generation time. Approximately 2×10^5 cells were ·seeded into 25-cm^2 flasks and viable cells were counted daily for 8 d. Cell counts were performed on a hemocytometer with trypan blue to determine viability. The logarithmic portion of the growth curve was used to calculate the generation time.

Clonogenic assays. Clonogenic assays were performed as described by Carney et al. (11). Briefly, cell pellets were resuspended in 0.4 ml RPMI 1640 with 10% FBS and were triturated. This suspension was mixed with 3.6 ml of a 0.3% agarose solution (Difco Laboratories, Inc., Detroit, MI) and 1-ml fractions were plated onto a bottom layer of 0.5% agarose in 35-mm Petri dishes (Falcon Labware, Oxnard, CA). Total colonies per plate were counted manually after 14 d.

MAbs. HNK-1 reacts with the Leu-7 determinant found on neural tissues, neuroblastoma cells, natural killer cells, and most SCLC but not NSCLC cells (12). HNK-1 has been shown to react with NCAM (13). W6/32 reacts with the HLA-A,B,C heavy chain and BBM.1 reacts with β_2-microglobulin (14). These three MAbs antibodies were obtained from the American Type Culture Collection, Rockville, MD. A rabbit antisera (Dako Corp., Santa Barbara, CA) specific for human β_2-microglobulin was also used. Antibodies AE-1, AE-2, and AE-3 were obtained from Dr. Tien-Treen Sun, New York University School of Medicine. AE-1 reacts against most acidic keratins and AE-3 reacts with all eight of the basic keratin species (15). AE-2 reacts with a 56,000-mol wt acidic keratin and 65,000- and 68,000 mol wt basic keratin species (15). Murine IgG1 MAbs UJ13A and AUA1 are reactive with SCLC cluster antigens 1 and 2, respectively, and were obtained from Dr. Robert Souhami, Courtauld Institute of Biochemistry, London, England (16). The cluster 1 antigen has been determined to be NCAM (6). Melanoma tumors and cell lines are only weakly stained by the cluster 2 antibody AUA1 and neuroblastoma is negative, but carcinoid tumors and cell lines are positive, showing, like SCLC, a mixture of neural and epithelial markers (16). A cDNA corresponding to this protein has been recently cloned and sequenced, revealing a transmembrane domain and several potential N-glycosylation sites (17). Murine IgG MAbs SWA22 and SWA20 react with SCLC cluster antigens 4 and 5, respectively, and were provided by Dr. Robert Waibel, University Hospital, Zurich, Switzerland (18).

Radioimmunoassay. The radiobinding assays were performed in 96-well polyvinyl-chloride microtiter plates (Costar Data Packaging, Cambridge, MA) as previously described (9). Adherent cells were harvested by scraping and single-cell suspensions were made by trituration through a 21-gauge needle. Target cells were fixed at a density of 10^5 cells per well with 0.25% glutaraldehyde and nonspecific protein binding to the wells was blocked with 1% bovine serum albumin in PBS. The plates were incubated with 5 μg/ml concentrations of primary antibody for 1 h, followed by incubation with a 1:100 dilution of affinity-purified rabbit anti-mouse Ig hyperimmune serum for 1 h (Jackson Research Laboratories, Bar Harbor, ME). All incubations were in PBS at room temperature. The detecting reagent was 40,000

cpm/25 μl of ^{125}I-labeled protein A (New England Nuclear, Boston, MA). Purified mouse myeloma IgG2a (RPC5, Litton Bionetics, Inc., Kensington, MD) was used as a negative control. Counting was performed on a Gamma 4000 counter (Beckman Instruments, Inc., Palo Alto, CA).

Analysis of indirect immunofluorescence by flow cytometry. Adherent cells were harvested by scraping and single-cell suspensions were made by trituration through a 21-gauge needle. After PBS washings, log-phase cultures of SCLC were incubated under saturation conditions with 1:100 to 1:500 dilutions of the protein A purified (2 mg/ml) antibodies. The total reaction volume was 0.2 ml. After incubation with shaking at room temperature for 1 h, the cells were pelleted at 1,500 g, washed with PBS, and incubated for 30 min with a 1:25 dilution of a fluorescein-conjugated goat anti-mouse IgG (Becton-Dickinson, Mountain View, CA). The cells were pelleted again, washed in PBS, resuspended in 0.2 ml PBS, and screened through a 43-μm wire mesh. The cells were then immediately assayed for mean fluorescence in a FACStar flow cytometer (Becton-Dickinson). 2.5×10^4 cells were analyzed using a live gate placed on forward light scatter to exclude cell debris and dead cells. 300 MW of laser power and 488-nm excitation light were used. Fluorescence emission was collected through a 530/30 filter. Percent positive cells and mean fluorescence were determined using the Consort 40 software package (Becton-Dickinson). The lines were also analyzed using a control irrelevant antibody, the murine IgG2a myeloma protein RPC5 or the murine IgG1 myeloma protein MOPC 21.

Preparation and hybridization of lung cancer cell line RNA. Total RNA was prepared from fresh cell cultures by the guanidium isothiocyanate/cesium chloride technique (19), and quantitated by absorbance at 260 nm. Quantitation was confirmed by electrophoretically fractionating a sample on formaldehyde agarose gels, staining with ethidium bromide, and observing the intensities of the ribosomal RNA bands. 20 μg of total RNA from each line was denatured and electrophoresed on a 1% agarose-formaldehyde gel (20) that had been modified by using 2.2 M formaldehyde in the gel and electrophoresing at 70 V for 5 h. The gels were transferred to nitrocellulose by capillary blotting and hybridization was performed using 50% formamide with the ^{32}P-labeled probe and washing at 65°C. The c-*myc* probe consisted of a 1.5-kb ClaI-*Eco*RI fragment encompassing the third exon of the c-*myc* gene (21). The c-*myc*–containing plasmid, PMC 41 3RC, was obtained from Dr. S. Tronick (22). The purified fragment was ^{32}P-labeled by random priming (23).

Cytokeratin extraction, electrophoresis, and Western blotting. Cytokeratins were extracted using a procedure modified from Wu and Rheinwald (24). Cells were washed in PBS, pH 7.4, and placed into a Wheaton hand homogenizer containing 5 ml of 20 mM Tris-HCl, pH 7.4, 0.6 M KCl, 1% Triton X-100, and the protease inhibitors 1 mM PMSF, 1 mM EGTA, and 1 mM EDTA at 4°C. The insoluble fraction was pelleted by centrifugation at 10,000 g for 15 min, resuspended in the same buffer, recentrifuged, and resuspended in PBS. Gel electrophoresis and Western blotting of cytokeratins were as previously described (25). Each lane contained the high-salt extract from 2×10^6 cells. Briefly, the blots were incubated with either AE-1, AE-2 or AE-3 primary antibodies in 3% normal goat serum (AE-1 or AE-3, 1:200; AE-2, 1:100) for 16 h at 4°C. After washing, peroxidase-conjugated rabbit anti-mouse Ig (Cappel) was added, followed by 3-3-diaminobenzidine hydrochloride (Sigma Chemical Co.) 1 mg/ml with 0.05% H_2O_2 to develop the peroxidase reaction for 3–5 min before quenching with water.

Cytogenetic analysis. Cells were exposed to colcemid at a final concentration of 0.03 μg/ml for 30 min while the cells were in a logarithmic growth phase. Cells growing in a monolayer were washed briefly twice with HBSS (Grand Island Biological Co.) and exposed to 0.25% trypsin-EDTA (Grand Island Biological Co.) for 2–3 min. All cells were spun down at 1,200 g for 5 min. Hypotonic swelling, fixation, chromosome preparation, and Quinicrine banding procedures were the same as previously described (26).

NSE assay. Cell pellets were homogenized in 10 vol of 10 mM Tris phosphate buffer containing 1 mM MgSO$_4$. The homogenate was cen-

trifuged and the soluble protein concentration of the supernatant was measured by the method of Lowry et al. (27). The NSE assay was performed using a competitive radioimmunoassay protocol as previously described (4).

Statistical analysis. The significance of differences noted between groups of cell lines by various assays was analyzed using a Kruskal-Wallis test.

Results

Growth characteristics and appearance of cells. The surface-adherent and floating subpopulations of H446 cells were successfully segregated into relatively pure populations with a progressively decreasing tendency to revert to the original mixed pattern of growth. The surface-adherent and floating subcultures were maintained in culture for > 18 mo (Fig. 1). The surface-adherent subline initially grew slowly, but became progressively more vigorous and fibroblastoid in appearance. The floating subline had a generation time of 42±4 h (SEM of duplicate cultures) and the surface-adherent subline had a generation time of 33±3 h. Each of the sublines cloned in soft agar with an approximate efficiency of 0.1% and these colonies were used to clone the sublines.

Expression of NSE. The floating aggregate H446 cells consistently had higher expression of NSE than did the surface-adherent H446 subline. When standardized for protein concentration, the H446 floating cells, in a representative assay, had 1,021 ng of NSE/mg of protein±48 ng/ml (SEM of quadruplicate cultures), compared with 379 ng of NSE/mg of protein±19 ng/ml in the surface adherent H446 subline and 41 ng of NSE/mg protein±8 ng/ml in a NSCLC line NCI H522. The difference in NSE between the floating aggregate and surface-adherent H446 sublines was significant with $P < 0.025$.

Cytogenetic analysis. Metaphase chromosomes from between 10 and 13 cells were analyzed for the floating, surface-adherent, and parent H446 cell lines. The distribution of chromosome number was virtually the same between the three groups. All three cell lines had the derivative chromosome no. 3 with a deletion in the short arm which overlaps the characteristic deletion of 3p seen in SCLC cells (28). Structural chromosome abnormalities found in common between floating, adherent, and parent lines included der(3), del(8)(p21), del(11)(q13), der(18) + (18;?)(18pter → 18q23::?), and del(21)(q22). Numerical chromosomal abnormalities linking

the three lines included four no. 1 and four no. 7 chromosomes. There were no significant or consistent structural abnormalities observed in distinguishing among the floating, surface-adherent, or parental sublines of H446.

Reactivity of SCLC cluster antigens. Flow cytometric analysis was performed using antibodies reactive with dominant SCLC surface antigens. Antibody UJ13A reacts with a 145,000-mol wt protein, found on SCLC and other neuroendocrine tumors but not NSCLC cells (16). This protein has been recently identified on SCLC cells as the sialoglycoprotein NCAM (6). The expression of this antigen was significantly greater in the floating subline than in the adherent subline (Table I) with $P < 0.025$. Very marked differences in reactivity were also seen with the AUA1 monoclonal antibody, which reacts with a 40,000-mol wt surface protein of unknown function, found on many epithelial tumors including SCLC (16). The floating aggregate subline of H446 had three to four times the percentage of positive cells with AUA1 as the surface-adherent subline (Table I). These differences in AUA1 immunoreactivity were significant with $P < 0.025$. MAbs SWA 22 and SWA 20, reactive with different surface antigens on SCLC and a variety of neural and epithelial tumors, were equally positive with both surface-adherent and floating sublines of H446 (17).

HNK-1 reactivity. Radioimmunoassays, using the HNK-1 MAb on fixed SCLC cells, consistently demonstrated that the floating H446 cells had at least five times the reactivity of the surface-adherent subline (Table II). These differences were significant with $P < 0.005$. The NSCLC line, H125, used as a control, had the predicted low HNK-1 reactivity. HNK-1 has been demonstrated to react with NCAM and myelin-associated glycoprotein (13). Control antibodies against invariant determinants of HLA and β_2-microglobulin did not discriminate between the adherent and floating sublines of H446 (Table II) although they demonstrated the previously described paucity of class I histocompatibility antigens in SCLC cells relative to NSCLC cells (29).

To establish that the difference in surface antigen expression of NCAM-related determinants was related to the adherent phenotype, rather than simple clonal selection, we independently reselected surface-adherent and floating sublines from the parent H446 line. Multiple relatively pure cultures of floating and monolayer H446 cells were derived, without cloning, over a period of 3 mo. Repeat flow cytometric analy-
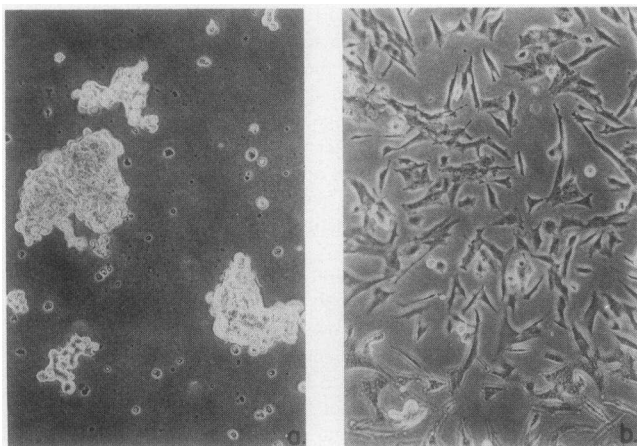

Figure 1. In vitro appearance of adherent and floating sublines of SCLC cell line NCI-H446: (*a*) floating; (*b*) surface adherent.

Table I. Effect of Surface-adherent or Floating Phenotype on Expression of SCLC Surface Antigens

	Percentage of positive cells	
MAb	H446 floaters	H446 adherent
UJ13A	20.0±1.1	3.6±0.4
AUA1	37.9±1.3	12.6±0.8
SWA 20	53.3±5.1	46.2±1.6
SWA 22	67.2±1.9	62.6±3.9
RPC5	1.8±0.6	5.4±0.3

Flow cytometric analysis of surface antigen expression on surface-adherent or floating sublines of H446. The percentage of positive cells represents the mean±SEM of duplicate cultures with 25,000 cells analyzed from each sample. RPC5 is an IgG2A murine myeloma protein used in all assays as a negative control.

Cell line	Antibody binding			
	NCAM MAb*	Anti–HLA MAb[‡]	Anti–β_2-microglobulin MAb[§]	Anti–β_2-microglobulin sera[∥]
		cpm ¹²⁵I-protein A/10⁵ cells		
H446-floaters	6,459±316	227±46	217±42	809±118
H446-adherent	1,286±87	163±39	246±35	691±74
H125 (NSCLC)	558±35	3,163±320	3,168±397	4,469±347

Solid-phase radioimmunoassay determination of radiobinding of antibodies to the surface-adherent and floating sublines of H446 and to the NSCLC line H125. Results are the mean of triplicate determinations±SEM. All antibody tests used a rabbit anti–mouse Ig connecting antibody followed by incubation with 40,000 cpm/25 μl of ¹²⁵I-staphylococcal protein A. * Antibody HNK-1 reacts with the Leu 7 determinant on NCAM. ‡ The W6/32 MAb reacts against an invariant determinant of the HLA-A,B,C heavy chain. § Antibody BBM.1 reacts against β_2-microglobulin. ∥ A rabbit anti–human β_2-microglobulin hyperimmune serum was also used. The experiment was performed three times with similar results.

sis, with UJ13A and HNK-1 antibodies, confirmed that the surface-adherent sublines were almost entirely nonreactive, while the floating sublines had 25–30% positive cells for UJ13A and 55–62% positive cells with HNK-1 (data not shown). Each of these differences were highly significant with $P < 0.005$.

We examined two other SCLC cell lines, which grow in vitro as a strongly adherent monolayer, to further investigate the link between low NCAM immunoreactivity and surface-adherent growth. Such SCLC lines, always of the variant subtype, are unusual but well described in the literature (1, 3). The two surface-adherent SCLC lines, H196-B and H1607, were nonreactive with either UJ13A or HNK-1, as was the surface-adherent NSCLC line H157 (Table III). The classic SCLC lines H209 and H146, and the SCLC-v line N417, each of which forms floating aggregates in culture, were positive for each antibody (Table III). The differences in both UJ13A and HNK-1 immunoreactivity between the three floating aggregate cell lines and the three lines forming monolayer surface-adherent cultures were highly significant, with $P < 0.005$ for each antibody.

Table III. Effect of Adherent or Floating Phenotype on Expression of NCAM Determinants

Cell line	Percentage of positive cells	
	UJ13A	HNK-1
H209 (classic SCLC)	16.3±1.6	47.9±2.3
H146 (classic SCLC)	35.2±0.7	54.1±2.2
N417 (variant SCLC)	27.6±1.1	68.6±2.1
H196-B (adherent SCLC)	5.5±0.2	1.0±0.2
H1607 (adherent SCLC)	0.6±0.1	0.7±0.1
H157 (NSCLC)	0.2±0.1	8.4±0.5

Flow cytometric analysis of NCAM determinants on lung cancer cell lines. The percentage of positive cells represents the mean±SEM of quadruplicate cultures with 25,000 cells analyzed from each sample. A 1:500 dilution of UJ13A and a 1:1,000 dilution of HNK-1 were used. MOPC 21, a control mouse IgG1 myeloma protein used as a negative control at a 1:500 dilution, had < 0.6% positive cells for each cell line. The experiment was repeated twice with similar results.

Keratin analysis. No significant differences were seen between surface-adherent and floating H446 cells in the pattern of intermediate filament immunostaining (Fig. 2). Both sublines showed a pattern of reactivity typical of variant SCLC cells (25, 30). The immunoblot results of floating and surface-adherent H446 cells were identical with respect to the known keratin molecular weight species between 40,000 and 68,000. Some minor lower molecular weight bands in the surface-adherent subline were noted, which may or may not represent some proteolysis of the keratins.

Analysis of c-myc expression. RNA extracted from the parent H446 line as well as the surface-adherent and floating sublines was analyzed by Northern blotting and hybridization with a labeled c-myc probe. The parent H446 cells had the expected high expression of c-myc, as did cells from the surface-adherent and floating sublines, neither of which varied
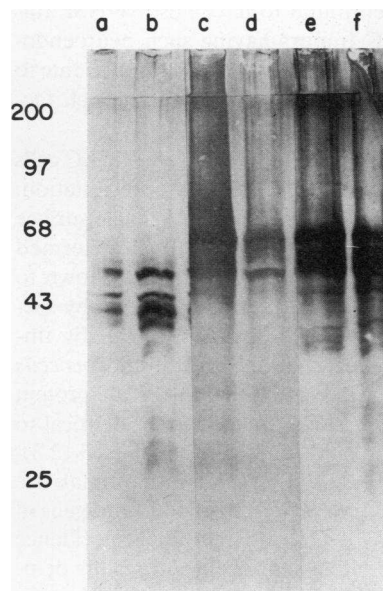

Figure 2. Immunoblots of three cytokeratin antibodies on cytoskeletal preparations from surface-adherent and floating sublines of the variant SCLC cell line NCI-H446. MAb AE-1 is reactive with most acidic keratin species. MAb AE-2 is reactive with 56,000- and 65,000-mol wt keratin species. MAb AE-3 is reactive with all eight basic keratins. (*a*) AE-1 immunoblotting of floating H446 cells; (*b*) AE-1 immunoblotting of surface-adherent H446 cells; (*c*) AE-2 immunoblotting of floating H446 cells; (*d*) AE-2 immunoblotting of surface-adherent H446 cells; (*e*) AE-3 immunoblotting of floating H446 cells; (*f*) AE-3 immunoblotting of surface-adherent H446 cells. Molecular weight markers shown at left are in thousands.
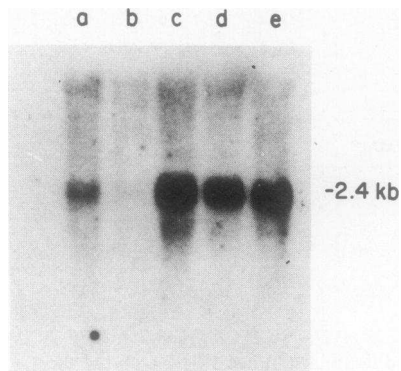
Figure 3. Northern blot analysis of c-*myc* expression of SCLC and NSCLC cell lines. Lane *a,* SCLC-v line NCI-H82; lane *b,* NSCLC line NCI-H125; lane *c,* the parent NCI-H446 line; lane *d,* the floating subline of NCI-H446; and lane *e,* the surface-adherent subline of NCI-H446.

significantly from the c-*myc* expression of the parent line (Fig. 3).

Discussion

One of the most obvious differences between SCLC and NSCLC cells in culture is that NSCLC cells typically grow as anchorage-dependent monolayers in vitro, whereas SCLC cells form multicellular floating spheroidal aggregates. The molecular differences in cell–cell aggregation and substrate adhesion between SCLC and NSCLC responsible for these differences have not been established. The unique SCLC line NCI-H446 provides an opportunity to compare phenotypes of adherent and floating lung cancer cells from a single genotype. The cytogenetic analysis strongly supports the identity of these sublines as SCLC and virtually eliminates the possibility that the adherent H446 cells might have resulted from contamination by foreign cells. The H446 subline that grew in an surface-adherent monolayer, similar to that of NSCLC, had a diminution of several neuroendocrine properties seen in SCLC cells. The expression of NSE in surface-adherent H446 cells is less than in the floating H446 cells, although not as low as the levels found in most NSCLC cells. A fraction of NSCLC tumors, however, have been found to have positive NSE immunostaining, and NSCLC tumors having such neuroendocrine features have been noted to have a higher response rate to combination chemotherapy than tumors without such factors (31).

Numerous MAbs reactive with SCLC but not NSCLC cells have been found by tissue distribution, immunoprecipitation, and competitive binding studies to react with a single surface protein of 145,000 mol wt (16). These antibodies, termed SC-1, include MAb UJ13A. SC-1 MAbs have been shown to recognize a SCLC protein identical to one recognized by specific polyclonal antiserum directed against NCAM, by immunoinhibition, immunoprecipitation, and *Staphylococcus aureus* V8 digestion studies, indicating that the SCLC protein bearing SC-1 determinants is closely related to or identical to NCAM (6). Long polysialic acid units composed of α-(2,8)-linked *N*-acetylneuraminic acid units, which in mammals are found exclusively on NCAM, were present on SC-1 antigens in SCLC and neuroblastoma (6). This provides further evidence that SC-1 MAbs recognize NCAM. Our current results demonstrate that binding of the SC-1 MAb UJ13A is significantly decreased in the H446 subline growing as a monolayer compared with the subline growing as typical floating SCLC spheroids. These floating spheroids demonstrate strong homophilic cell–cell adhesion that may be mediated by NCAM.

SCLC cell lines and biopsies have been shown immunocytochemically to express an antigen recognized by HNK-1, a mouse MAb which recognizes a surface antigen on natural killer cells and on NCAM and the myelin-associated glycoprotein (12, 13). NSCLC cells have little or no reactivity with the HNK-1 antibody. Recent studies have shown that HNK-1 recognizes a carbohydrate determinate on a variety of neuroectodermal tumors including glioblastomas, astrocytomas, pheochromocytomas, and medullary thyroid carcinomas (32). By Western blotting, HNK-1 recognizes the 180- and 140-, but not the 120-kD band of NCAM isolated from adult mouse brain by immunoaffinity purification, using another MAb BSP-2 (13). The HNK-1 epitope appears to be a carbohydrate moiety, in that it is heat resistant, destroyed by periodate treatment, but resistant to pronase (13). Immunoblot data on SCLC cells identify two groups of HNK-1–reactive plasma membrane glycoproteins of 80,000 and 130,000 mol wt (33). SCLC cells also release a 100,000-mol wt glycoprotein reactive with HNK-1 into the spent medium of cultured cells (34). A secreted form of NCAM has also been demonstrated in neural tissues (35).

Our results indicate that HNK-1 is a second NCAM-directed MAb which is markedly less reactive with the surface-adherent H446 subline relative to the floating subline. Our finding that the ratio of binding of cells from floating compared to surface-adherent H446 sublines is approximately 5, with both HNK-1 and UJ13A antibodies, suggests that these two antibodies recognize the same antigen on SCLC cells.

We have shown that two other unusual SCLC-v cell lines, which grow as surface-adherent cell lines and have low expression of neuroendocrine markers, do not react with UJ13A or HNK-1. These findings generalize the association of NCAM immunoreactivity with SCLC growth as floating aggregates. In addition, up to 12% of NSCLC cancers have features of neuroendocrine differentiation (31). Cell lines established from these tumors are unusual for NSCLC in that the cells grow as floating aggregates and have been demonstrated to react with HNK-1 and another NCAM-directed MAb NKH1 (36). This observation further strengthens the association of NCAM immunoreactivity and the adhesive properties of lung cancer cells and suggests that NCAM expression closely correlates with other markers of neuroendocrine differentiation.

The greater UJ13A and HNK-1 reactivity of floating H446 cells, compared to the H446 adherent cells is the first reported association between NCAM-related determinants and homophilic cell–cell adhesion between SCLC cells. The association is strengthened by other findings we have reported with SCLC-v cells incubated with retinoic acid (37). These retinoid-treated cells have a marked phenotypic change with the loosely aggregated variant cells becoming much more tightly clustered spheroids. This increased cell–cell adhesion is associated with a marked increase in HNK-1 reactivity among the retinoid-treated cells compared with untreated control cells (37).

Other factors beside the total amount of NCAM protein may be responsible for the different adhesive properties of the floating and adherent H446 cells. Polysialylation of NCAM has been demonstrated to markedly reduce the homophilic binding of neural cells (38). Different NCAM isoforms, owing to alternative RNA splicing, could also potentially change the adhesive properties of the molecule (39). We are currently investigating whether there are differences in sialylation of

NCAM proteins between the H446 sublines. In addition, the SCLC lines that grow as adherent monolayers may have an altered interaction with laminin, fibronectin, or other basement membrane proteins, relative to the more common SCLC lines that grow as floating aggregates in vitro. SCLC cells have laminin receptors and bind to laminin in adherence assays (40). However, the inability of most SCLC cells to form monolayer cultures may be related to their lack of detectable fibronectin receptors, and weak binding to fibronectin in vitro (41).

Recent evidence, including keratin patterns, suggest that SCLC derives from an epithelial bronchial cell rather than from cells which have migrated from the neural crest (42). Our finding that the adherent H446 cells retain a SCLC-v keratin pattern suggests that there is no increase in terminal differentiation in an epithelial direction of these surface-adherent cells despite the morphologic changes. It appears, instead, that the adherent H446 have not undergone the neuroendocrine differentiation characteristic of most SCLC cells. Neuroendocrine differentiation of bronchial cells in SCLC could explain the appearance of the surface adhesion molecule NCAM. There is evidence that NCAM is a functionally important molecule in cell–cell interactions and adhesion in the developing nervous system (43). NCAM may potentially be important in the early metastasis characteristic of SCLC cells. It has already been demonstrated, for example, that the 20% of patients with NSCLC whose tumors were positive for NCAM had a significantly shorter survival than did NSCLC patients with tumors negative for NCAM immunoreactivity (7). The adherent and floating sublines of H446, which we have developed, should be useful models to study the expression and modulation of NCAM in SCLC cells.

Acknowledgments

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