Differentiation of Human Variant Small Cell Lung Cancer Cell Lines to a Classic Morphology by Retinoic Acid

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ABSTRACT

Variant small cell lung cancer (SCLC) is distinguished from the classic histology by changes in growth characteristics and morphology, c-myc amplification, a loss of some biochemical markers, and relative chemoresistance. Three variant SCLC lines were incubated in 1 μM all-trans retinoic acid. After 8–10 days, a marked change in morphology was noted in all three lines, with tight cell aggregation and central necrosis of large floating spheroids similar to classic SCLC. The retinoid-treated cells demonstrated decreases in growth rate and cloning efficiency to levels comparable with classic SCLC cell lines. Retinoic acid incubation caused a reproducible decrease in c-myc expression in variant SCLC cells, but was not noted to increase L-dopa decarboxylase, an enzyme which biochemically distinguishes classic from variant SCLC cell lines. Retinoid exposure led to an increase in HLA and Leu-7 antigens, but a reduction of antibody binding to 3-fucosyllactosamine, a dominant SCLC glycolipid antigen. Clonogenic assays revealed that the variant cells, after incubation in retinoic acid, became more sensitive to etoposide, but more resistant to Adriamycin. We conclude that exposure of variant SCLC cells to retinoic acid can lead to a morphologic phenotype similar to classic SCLC, but with substantial differences in cell biology.

INTRODUCTION

SCLC3 comprises 20–25% of bronchogenic carcinomas, and is distinctive in its biochemical, pathological, and clinical behavior (1). An important subset of SCLC are the SCLC variant tumors, which can usually be distinguished from classic SCLC by morphology alone. Variant SCLC may pathologically resemble large cell undifferentiated carcinoma, with large cells and prominent nucleoli distinguishing the tumor from classic SCLC (2). Approximately 30% of SCLC cell lines established will have variant features, with small floating cords of loosely adherent cells distinguishing the variant cells from the centrally necrotic and tightly compacted spheroids characteristic of type 1 classic SCLC cultures (3). Variant SCLC cells typically lack DDC expression and bombesin-like immunoreactivity, but retain expression of the SCLC markers neuron-specific enolase and the brain isofrom of creatine kinase (4, 5). Variant SCLC cells have shorter doubling times and a higher cloning efficiency than classic SCLC cells (3) and frequently manifest amplification of the c-myc oncogene (6). The variant subtype is associated with radioresistance in vitro and a more virulent progression in patients compared to classic SCLC (7).

Since many variant SCLC cell lines originate from tumors with a variant morphology and since the biochemical and morphological characteristics are distinct within the first weeks of tissue culture, it is likely that the alterations found in variant SCLC lines reflect changes that had already occurred in the tumors from which they were derived (2). As spontaneous transformation of a cloned classic SCLC line to a variant phenotype has been documented and since variant cell lines are usually established from metastatic sites late in the natural history of the disease, it is likely that variant SCLC represents a progression of cancer from an original classic SCLC lesion (3). Since variant SCLC cells have a less differentiated neuroendocrine phenotype than their classic counterparts, it was of interest to us to examine the effect of a differentiation inducer on variant SCLC cells.

Retinoids, the family of molecules comprising both the natural and synthetic analogues of retinol, are potent agents for control of both cellular differentiation and proliferation (8). Vitamin A is important in lung development and may have a role in the prevention of lung cancer (9). Deficiency of vitamin A has been reported to induce squamous metaplasia in hamster trachea (10). In addition, retinoic acid can effect regression of premalignant lung lesions and can also exert effects on certain fully transformed, invasive cancer cells (11). While cellular retinoic acid binding protein has been found in the cytosol of most NSCLC specimens, it has not been detected in SCLC specimens (12, 13). Nevertheless, Olsson et al. (14) noted profound effects of RA on the growth, cloning, and tumorigenicity of both SCLC and NSCLC cells. Francis et al. (15), in similar experiments, demonstrated only a slight antiproliferative effect of RA on a classic SCLC line and no change in DDC expression or colony morphology. To investigate these divergent findings and to explore possible parallels with the reduction of N-myc in neuroblastoma cells by RA (16) we examined the effect of this agent on variant SCLC cells in culture.

MATERIALS AND METHODS

Materials. For cell culture, RPMI 1640, glutamine, and fetal calf serum were purchased from Grand Island Biological Co., Grand Island, NY, L-[14C]dopa, 8 mCi/ml, used for assays of DDC activity, was purchased from Amersham Searle, Arlington Heights, IL; Retinoic acid (all-trans) was obtained from Sigma Chemical Co., St. Louis, MO; etoposide was from Bristol Laboratories, Syracuse, NY; doxorubicin was from Adria Laboratories, Columbus, OH; agarose was from Sea Kem, Rockland, MA; [3H]dCTP, 3000 Ci/ml, and °I-labeled-protein A, 30 mCi/μg, were from New England Nuclear, North Billerica, MA; insulin, transferrin, selenium, estradiol, and hydrocortisone, were all from Collaborative Research Inc., Lexington, MA.

Monoclonal antibody 9456-SA (Bethesda Research Laboratories, Gaithersburg, MD) reacts with an invariant determinant of the HLA-A,B,C antigen. Monoclonal antibodies BBM.1, reactive with β₂m and HNK-1, reactive with the Leu-7 determinant were obtained from the American Type Culture Collection, Rockville, MD. Monoclonal antibody 534-F8, reactive to 3-fucosyllactosamine, was obtained from Dr. F. Cuttitta (17). A human 1.5-kilobase DNA fragment encompassing the third exon of the human c-myc gene was obtained from Dr. S. Tronick (18). The c-myc fragment pMC41 3RC was removed from pBR322 by double digestion with Clal and EcoRI endonucleases (19).

Cell Culture Conditions. The variant SCLC lines NCI-H82, NCI-N417, and NCI-H526 have been previously described (3). Each cell line

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3 The abbreviations used are: SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer; RA, retinoic acid; DDC, L-dopa decarboxylase; dAMP, dithyryl cyclic adenosine 3′,5′-monophosphate; β₂m, β-2 microglobulin.

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forms tumors in nude mouse heterotransplants with a histology consistent with variant SCLC and has elevated neuron specific enolase and creatine kinase biomarkers. Lines N417 and H82 have amplification of the c-myc oncogene while H526 has an amplified N-myc gene. Cell lines H526 and H82 were derived from patients with an initial variant SCLC histology, while line N417 converted from a classic to variant SCLC phenotype in vitro. The classic SCLC cell line NCI-H592 forms tumors in nude mouse heterotransplants with a classic SCLC histology, and differs from the variant SCLC lines by having detectable DDC and bombesin-like immunoreactivity (3). All lines exhibit human enzymes by starch gel electrophoresis (17) and were grown in RPMI 1640 medium supplemented with 2% fetal calf serum and HITES reagents (hydrocortisone, insulin, transferrin, estradiol, and selenium) as described (20). Cells were seeded into 25-cm² flasks (Falcon) at initial densities of 1-2 × 10⁵ cells/ml and incubated with 1 µM all-trans-retinoic acid. Viable cells were identified from cell culture by trypan blue exclusion. RA was dissolved in 95% ethanol to make a 1 mM stock solution and was diluted into the growth medium to a final concentration of 1 µM RA and 0.1% ethanol. Control flasks only received growth medium with 0.1% ethanol. The medium in control and RA-treated cultures was changed every 4 days.

Clonogenic Assays. The effect of RA on the cloning efficiency of variant SCLC was tested by using a soft agarose clonogenic assay as described previously (21). Briefly, cells in log phase were harvested, tritutated, and washed twice in serum-free medium immediately prior to assay, then mixed with 0.3% (w/v) agarose in culture medium and plated in 35-mm Petri dishes over a preformed base layer of 0.5% agarose and culture medium. The number of cells plated was chosen so that an estimated 500 colonies would be obtained in control dishes. After 14 days, plates were scored for colony growth (cell aggregates of greater than 50 cells) with an inverted phase microscope. All studies were done in triplicate and each point represents the mean colony count.

In radiation studies, cells were suspended in a 0.5-ml volume and irradiated at room temperature by using a 100-kV (peak) photon beam from a General Electric X-ray unit. The dose rate was 5.0 Gy/min. Following irradiation, the cells were tritutated into single cell suspensions, counted, and plated at varying densities in soft agarose as described above. In vitro chemosensitivity studies were as previously described (7). Cells were exposed for 1 h at 37°C to increasing concentrations of doxorubicin or etoposide. The concentrations tested included the peak achievable human plasma level of the drugs and 1- and 2-log dilutions of that level. After incubation, the cells were washed twice by centrifugation in serum-free medium and plated in soft agarose as described above. In plates containing drug-treated cells, the number of clonogenic cells surviving treatment was expressed as a percentage of control.

Radioimmunoassay. The radiobinding assays were performed in 96-well polyvinyl microtiter plates (Costar, Cambridge, MA) as previously described (22). Target cells were fixed at a density of 10⁵ cells/well with 0.25% glutaraldehyde and nonspecific binding to the wells was blocked with 1% bovine serum albumin in phosphate-buffered saline. The plates were incubated with antibody for 1 h, followed by a second incubation with affinity-purified rabbit anti-mouse hyperimmune serum (Jackson Immuno-Research, Avondale, PA) for 1 h. All incubations were in phosphate-buffered saline at room temperature. The detecting reagent used was 40,000 cpm of ¹²⁵I-labeled staphylococcal protein A. Purified, class-matched mouse myeloma proteins (Litton Bionetics, Inc., Kensington, MD) were used as negative controls.

1-Dopa Decarboxylase Assay. DDC activity was determined on 80-µl cell homogenates by using a previously described modification of the method of Baylin et al. (23) and Bean et al. (24). The activity is expressed as units/mg protein, where 1 unit is 1 µmol ¹⁴C deaminated/h of incubation. Protein determinations on all samples were performed by the method of Lowry et al. (25).

Preparation and Hybridization of Lung Cancer Cell Line RNA with a c-myc Probe. Total cellular RNA was isolated by lysis in guanidine isothiocyanate as described by Cox (26) and Strohman et al. (27) and quantitated by absorbance at 260 nm. Twenty µg of total cytoplasmic RNA was denatured and electrophoresed on a 1% agarose-formaldehyde gel (28) that had been modified by using 2.2 M formaldehyde in the gel and electrophoresing at 70 V for 5 h. The gel was transferred to nitrocellulose by capillary blotting. Blots were probed with an EcoR1-Clal fragment from PMC41 3RC, which was labeled with ³²P to an activity of 9 × 10⁶ cpm by oligolabeling (18, 29). Hybridization was performed with 50% formamide and the blots were washed at 65°C (30).

RESULTS

Effects of RA on Growth of SCLC Cells. A 1 µM concentration of RA had no effect on the morphology or viability of the classic SCLC cell line H592. When three variant SCLC cell lines (H526, H82, and N417) were incubated with 1 µM RA, each exhibited a marked change in colony morphology after 8–10 days of treatment, with the original loosely aggregated cells forming tight spheroids with central necrosis (Fig. 1). In the terminology suggested by Carney et al. (3), each line had converted from a type 3 to a type 1 SCLC morphology characteristic of classic SCLC lines.

The effect of 1 µM RA on the growth of the classic SCLC line H592 and variant lines H82 and N417 over a 10-day exposure was determined (Fig. 2). Control flasks for each line without differentiating agent were run in parallel, as were flasks containing 1 µM dCAMP and 1 mM theophylline, recently found by Francis et al. (15) to cause cessation of SCLC growth. RA had no effect on the growth of classic H592 cells which remained 98% viable after 12 days of culture (Fig. 2A). RA had a marked cytostatic effect on the variant lines H82 (Fig. 2B) and N417. In addition, some cytotoxicity was evident since the percentage of viable cells in the N417 line at day 10 dropped from 70% in the control flask to 38% in the RA-containing flask. The percentage of viable cells in the H82 line dropped from 90% in the control flask to 66% in the RA-containing flask.

In contrast, the combination of dCAMP and theophylline had marked antiproliferative effects on both classic and variant SCLC lines. The effect at day 10 appeared cytostatic for the classic line H592 and cytotoxic for two variant lines which had a 50–75% reduction in cell numbers and only 7–8% viable cells.

We next examined the persistence of retinoid-induced changes in SCLC variant morphology and growth after removing the differentiating agent. The classic SCLC line H592 and the variant line H82 were grown in the presence of 1 µM RA for 13 days, then the cells were washed and grown in the absence of RA. Control flasks of H592 and H82 untreated with RA were also observed and counted. After 10 days of observation the H82 cells removed from RA-containing medium gradually reverted from classic to a type 3 variant morphology. After a lag period of 5–6 days the cells began to proliferate (Fig. 3). H82 cells kept in RA-containing medium continued to have a classic SCLC type 1 morphology and a markedly reduced growth rate (Fig. 3). The classic SCLC H592 cells, whether remaining in RA-containing medium or not, continued to grow as fast as H592 cells never exposed to the agent.

Variant H82 cells were grown in the presence of varying concentrations of RA ranging from 10⁻⁴ to 10⁻¹ M. No significant effects on cell growth or colony morphology were noted at 10⁻⁴ or 10⁻³ M concentrations. However, a partial effect on phenotypic conversion and proliferation was observed with 10⁻⁷ M RA, after a 10-day treatment, with larger, more tightly aggregated colonies noted, as well as slower growth and a 65% decrease in cloning efficiency.

Cloning Efficiency of SCLC Cells after Exposure to RA. Clonogenic assays were performed with cells from two variant...
SCLC lines, N417 and H82, and the classic SCLC line H592 (Table 1). After 11 days of growth in RA-containing media, washed single cell suspensions were plated into agarose plates not containing RA. Colony counts at day 14 revealed that both the variant and classic SCLC cells previously treated with RA cloned approximately 40% as efficiently as control cells never exposed to RA. There were no significant differences in colony size or appearance between colonies from any cell line previously grown with or without RA.

An alternative cloning scheme determined colony-forming efficiency of SCLC by seeding previously untreated cells into agarose containing 1 μM or 0.1 μM RA. Cloning of the variant SCLC line H82 was decreased 60–70% by direct cloning in RA-containing agarose and variant line N417 cloning was decreased 30–40% (data not shown). The effect on cloning efficiency of variant SCLC cells was seen equally with 1 μM and 0.1 μM concentrations of RA. In contrast, the cloning of the classic line H592 was stimulated over 50% by 0.1 μM RA.

Effects of RA on Specific Activity of DDC. The relationship of DDC expression to changes in growth and morphology of variant SCLC cells by RA was explored. Cell homogenates were prepared from variant lines H82, H526, and N417, and the classic line H592 after 11 days of exposure to 1 μM RA. Control lysates from untreated cells were also prepared. Measurements of DDC specific activity in the variant lines indicated that morphologic conversion of cells to a classic SCLC appearance was not associated with the induction of DDC expression. The high DDC specific activity of the classic H592 cells was not appreciably changed by incubation in RA. Cells from a non-small cell lung cancer line, NCI-H157, were negative for DDC expression as expected.

Effect of RA on Variant SCLC Surface Antigens. The surface expression of HLA-A,B,C, β2m, and Leu-7, and 3-fucosyllactosamine determinants were assayed on variant SCLC cells before and after incubation with 1 μM RA (Table 2). A paucity of HLA-A,B,C and β2m antigen expression has been noted in SCLC cells, with variant SCLC cells displaying even lower expression of these antigens than classic SCLC cells (31). The Leu-7 determinant, defined by binding of monoclonal antibody HNK-1, is positive on SCLC cells in contrast to NSCLC cells (32). HNK-1 reactivity is usually greater in classic than in variant SCLC lines. The determinant 3-fucosyllactosamine, defined by reactivity to monoclonal antibody 534F8, is found on all SCLC cells, but is more heavily expressed on classic than on variant lines (17).

Radiobinding assays determined that in the classic SCLC line H592 and the variant lines H82 and N417, exposure to 1 μM RA for 2 weeks effected a 3- to 5-fold induction of HLA-A,B,C and β2m surface antigens compared to untreated control cells (Table 2). HNK-1 reactivity also increased in both classic and variant lines by 2- to 10-fold. Radiobi binding studies with the 534F8 monoclonal antibody determined that RA treatment of variant cell lines H82 and H526 decreased the reactivity of the cells with this antibody. The latter finding was unexpected, in that the assumption of variant cells of a classic colony morphology did not correlate with the high radiobi binding of 534F8 seen with true classic cell lines.

Expression of c-myc in Variant SCLC Cells after RA Incubation. Steady-state levels of c-myc mRNA were examined in the variant SCLC line H82 at various times after RA exposure, using the human c-myc probe pMC41 3RB (18). A reduction in c-myc levels was seen in the RA-treated cells by 8 h, compared to matched control cells (Fig. 4). This reduction in c-myc persisted to 72 h. Some diminution in c-myc levels was noted in the control H82 cells after 24 h, presumably due to the cells entering a stationary growth phase, but a greater reduction in c-myc levels was seen in the RA-treated cells at each time point relative to control cultures. After a 2-week exposure to 1 μM RA, with refeeding every 4 days, line H82 reproducibly demonstrated almost undetectable c-myc steady-state levels compared to high expression in control H82 RNA (data not shown). Therefore, progressive reductions in c-myc expression precede the phenotypic conversion of a variant SCLC cell line to a classic morphology.

In Vitro Radiation and Chemotherapy Sensitivity of RA-treated Variant Cell Lines. Variant SCLC has been associated with a poor response to cytotoxic chemotherapy clinically and has been shown to be radioresistant in vitro compared to classic SCLC cells (7). The variant SCLC line H82 was used to assay whether a 2-week incubation in 1 μM RA would alter the relative
Differentiation of Variant SCLC

TREATMENT PERIOD (DAYS)

CONTROL
RA
dcAMP

CONTROL
RA DISCONTINUED
RA CONTINUED

Viable cells x 10^5

Fig. 2. The classic SCLC line H592 (upper) and variant SCLC line H82 (lower) were grown in control medium with 2% fetal calf serum and HITES reagents (hydrocortisone, insulin, transferrin, estradiol, and selenium) or with control medium plus 1 μM RA; or with control medium plus 1 μM dcAMP plus 1 mM theophylline. Total and viable cells were counted every other day and refed every 4 days with fresh medium and differentiating agents.

Fig. 3. Variant SCLC line H82 was grown in control medium with or without 1 μM RA for 13 days, then washed and placed in fresh medium with or without 1 μM RA at day 0 of cell counting. • cells grown in control medium followed by further control medium, ▲ cells grown in RA-containing medium, followed by continued growth in RA-containing medium at day 13; ▲ cells differentiated with RA-containing medium, then washed and grown in medium not containing RA at day 13. Cells were counted every other day and refed every 4 days during the experiment with the same medium as at day 0.

Table 1 Cloning efficiency of SCLC cells after treatment with RA

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Mean no. of colonies</th>
<th>Cloning efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>82</td>
<td>1139</td>
<td>1.82</td>
</tr>
<tr>
<td>82-RA</td>
<td>488</td>
<td>0.78</td>
</tr>
<tr>
<td>417</td>
<td>854</td>
<td>1.37</td>
</tr>
<tr>
<td>417-RA</td>
<td>318</td>
<td>0.51</td>
</tr>
<tr>
<td>592</td>
<td>159</td>
<td>0.24</td>
</tr>
<tr>
<td>592-RA</td>
<td>67</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Table 2 Radiobinding of monoclonal antibodies (MoAbs) to SCLC surface antigens after RA treatment in solid phase radioimmunoassays

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Binding of MoAbs (cpm 125I-labeled protein A bound over background/10^5 cells) against</th>
</tr>
</thead>
<tbody>
<tr>
<td>9456-SA</td>
<td>82  172                        382                        376                        6753</td>
</tr>
<tr>
<td></td>
<td>82-RA  337                     958                        1075                       3872</td>
</tr>
<tr>
<td></td>
<td>417   331                      883                        5662                       NT*</td>
</tr>
<tr>
<td></td>
<td>417-RA 2650                   2005                       9189                       NT</td>
</tr>
<tr>
<td></td>
<td>526   NT                       NT                         83                         6016</td>
</tr>
<tr>
<td></td>
<td>526-RA NT                      NT                         1114                       2981</td>
</tr>
<tr>
<td></td>
<td>592   433                      422                        3424                       NT</td>
</tr>
<tr>
<td></td>
<td>592-RA 2153                    3706                       7759                       NT</td>
</tr>
</tbody>
</table>

* NT, not tested.

Radio- and chemoresistance of that line. The patient from which H82 was established had not received radiation therapy, but had been treated with vincristine, doxorubicin, 1,3-bis(2-chloroethyl)-1-nitrosourea and methotrexate. The parental H82 line has been previously studied (7) and been found to be relatively resistant to these chemotherapy agents.

Prior exposure of H82 cells to RA for 10 days led to a reproducible increased sensitivity of the cells to a 1-h incubation of cells with the peak achievable plasma level of etoposide (Table 3). Cells were washed after exposure to etoposide and plated in agarose containing neither etoposide nor RA. Similar assays indicate that RA-treated H82 cells are paradoxically more resistant to higher concentrations of doxorubicin than control H82 cells not exposed to RA (Table 3). Incubation of H82 cells with RA was not associated with any change in the extrapolation number (n) of the X-ray survival curves. The in vitro response of both parental and RA-exposed H82 cells approximated the previously reported results (data not shown).

Variant H82 cells were also exposed to varying concentrations of etoposide for 90 min, washed, and then plated into agarose containing either 0, 0.1, or 1 μM RA. This sequence of drug exposure also demonstrated a supraadditive decrease in cloning of cells exposed to both etoposide and RA (data not shown). An effect was noted with 0.1 μM RA upon cells treated

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with 0.6 to 17 μg/ml of etoposide, although the enhanced cytotoxicity was less profound than when cells were plated in agarose containing 1 μM RA.

**DISCUSSION**

The present studies extend our knowledge concerning the relationships between classic and variant subtypes of SCLC. In cell culture and in patients affected with the disease, SCLC cells appear to be capable of progressing from a classic phenotype with pronounced neuroendocrine features to a faster growing, less differentiated variant subtype. We have shown that RA treatment can reverse some features of this progression. Treatment of variant SCLC cell lines with 1 μM RA causes the cells to assume a morphology similar to that of classic SCLC cells. These RA-treated variant cells also have slower doubling times in vitro and lower cloning efficiencies in agarose.

Etoposide (μg/ml) | 0 | 1.2 | 6 | 30 | 150 |
---|---|---|---|---|---|
Surviving fraction | 1.00 | 0.94 | 0.67 | 0.57 | 0.003 |

Table 3 Resistance of RA-treated SCLC cells to doxorubicin and etoposide

Ventricular SCLC line H82 was grown for 2 weeks with or without the addition of 1 μM RA. Cells from each group were exposed to varying concentrations of doxorubicin or etoposide for 1 h at 37°C. The cells were washed and triturated into a single cell suspension and 25,000 cells were plated in triplicate in soft agarose. The surviving fraction is calculated from the mean number of colonies counted after 2 weeks compared to the control plates of 82 and 82-RA cells not exposed to cytotoxic agents. Each result is typical of the results of at least 3 clonogenic assays.

Etoposide (μg/ml) | 0 | 0.005 | 0.05 | 0.5 |
---|---|---|---|---|
Surviving fraction | 1.00 | 0.69 | 0.55 | 0.11 |

Doxorubicin (μg/ml) | 0 |
---|---|
Surviving fraction | 1.00 |

**Table 3 Resistance of RA-treated SCLC cells to doxorubicin and etoposide**

FIG. 4. Expression of c-myc mRNA in a SCLC cell line at various times between 1 and 72 h after RA incubation. The variant SCLC line NCI-H82 was grown with or without 1 μM RA. Northern blot analysis was performed on 20 μg total cytoplasmic RNA samples which were denatured and electrophoresed on a 1% agarose-formaldehyde gel. The c-myc probe used was a Chl-EcoRI fragment from pMC41 3RC encoding the third exon of the c-myc gene. The lanes represent paired time points of RNA extracted after exposure to RA with 0.1% ethanol or control lanes (C) treated with 0.1% ethanol alone.

**DISCUSSION**

The combination of SCLC and squamous or adenocarcinoma components is seen occasionally in clinical biopsies (35). Gazdar et al. (36) have reported multipotent SCLC cells in vitro, showing simultaneous multidirectional differentiation without any treatment. The possession of these cells of dual neuroendocrine and epithelial characteristics supports the hypothesis that SCLC originates from a stem cell in the endoderm capable of multiple differentiation pathways (37). There is recent evidence that exposure to retinoids may influence the differentiation of SCLC cells. Terasaki et al. (38) found that removing vitamin A from the medium of cultured SCLC cells caused squamous differentiation of some SCLC cells as evidenced by changes in morphology, keratin pattern, and in the development of desmosomes by electron microscopy. These changes reverted to the original small cell morphology within 4 weeks after addition of RA to the medium. It is well established that vitamin A deficiency can cause squamous metaplasia of bronchial cells (10). It is reasonable to conjecture that some variant SCLC cells are capable of differentiating in either a squamous or a neuroendocrine pathway and that retinoids are important mediators of which differentiation pathway the cell will commit to.

The induction of differentiation-specific genes under RA control has been shown to differ from the effects of this agent on c-myc expression and growth control in many systems (8). Therefore, it is not surprising that the RA-treated variant SCLC cells did not assume the complete biochemical phenotype of classic SCLC. Some changes seen in the variant SCLC cells are similar to mouse melanoma cells, where RA causes tighter coupling between cells (39). Retinoid treatment of mouse neuroblastoma cells causes growth inhibition, lower saturation density, and a greater dependence on serum factors (40). While changes in serum can sometimes alter the growth and appearance of SCLC cells in culture, Carney, et al. and Gazdar, et al. have reported no changes in morphology or biochemical properties of large numbers of SCLC cell lines run in parallel in either serum-free or serum-supplemented medium (3, 4). The effects of RA on the three SCLC variant lines we tested are marked, reproducible, and independent of the concentration or lot of serum which remained constant throughout these experiments.

SCLC tumor specimens in contrast to NSCLC specimens, have not been found to have RA-binding proteins in prior studies (12, 13). Retinoids are known, however, to have differentiating effects on the HL60 promyelocytic leukemia line and 10T½ fibroblast cells, although these lines do not have detectable RA-binding proteins (41). The expression of the newly discovered retinoic acid receptor, which belongs to the family of steroid and thyroid hormone receptors, is not known in either SCLC or NSCLC cells, but probably has a more central role.
role in the differentiation process than do the RA-binding proteins (42, 43).

The antiproliferative and morphological effects of RA are probably related to changes in the activity of the c-myc oncogene. Johnson et al. have reported that transfection of the c-myc protooncogene into a classic SCLC line caused the transfected clones to develop a loosely aggregated morphology resembling variant SCLC (44). Clones with a stably integrated c-myc-containing plasmid had increased c-myc transcription, decreased doubling time, and increased cloning efficiency proportional to the copy number of inserted c-myc genes, but no decrease in the specific activity of DDC. In HL60, F9 teratocarcinoma cells and mouse neuroblastoma cells, steady-state c-myc or N-myc levels are regulated by RA, with posttranscriptional control mechanisms of great importance, and this down regulation precedes changes to the proliferative state of the myc-amplified cell line (45, 46). However, a human colon cancer line COLO 320, which has c-myc amplification and neuroendocrine properties, has no down regulation of c-myc with up to 10^5 M RA, although moderate decreases in cell proliferation are noted (47).

The maintenance of the classic morphology requires continuous exposure of variant cells to 1 μM RA. The effects persist for up to 3 months in RA-containing medium, but revertants with loosely aggregating cells and more rapid proliferation have been noted after this period.

Francis et al. (15) have reported on the effects of dAmp on growth of classic SCLC cells and the specific activity of DDC. This agent produces simultaneous cessation of cell growth and an increase in cellular DDC. The butyrate moiety can produce the inhibition of cell growth, but the elevation in DDC activity and n-myc oncogenes in human lung cancer. Cancer Res., 43: 2806–2811, 1983.


34. Gazdar, A. F., Carney, D. N., Russell, E. K., Sims, H. L., Baysin, S. B.,


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