Response of Primary Human Lung Carcinomas to Autocrine Growth Factors Produced by a Lung Carcinoma Cell Line

Jill M. Siegfried and Sara E. Owens

Carcinogenesis and Metabolism Section, Environmental Health Research and Testing, Inc., Research Triangle Park, North Carolina 27709

ABSTRACT

Medium conditioned for 48 to 72 h by A549-1 lung carcinoma cells was used to culture primary solid lung tumors on feeder layers of inactivated Swiss 3T3 cells. Of 22 cases placed into culture, primary cultures of carcinoma cells were obtained in 20. Subcultures were obtained in 18 cases, and cell lines were established in nine cases. The neoplastic origin of the cultured cells was demonstrated by several criteria: tumorigenicity in athymic mice; anchorage-independent growth; expression of altered lactate dehydrogenase isoenzyme profiles; and expression of the lung tumor marker pregnancy-specific glycoprotein 1. The epithelial nature of cultured carcinoma cells was demonstrated by expression of keratin. These characteristics were compared to normal epithelial cells established in culture from bronchial explants from the same donors as tumor tissue, or other donors. The growth-stimulating effect of conditioned medium toward primary or newly cultured tumor cells was quantitated by clonal assays in soft agar and in monolayer culture. Growth response in clonal assays of newly cultured carcinoma cells to the purified growth factors transforming growth factor α and insulin-like growth factor 1, two known components of medium conditioned by A549-1 cells, was also demonstrated.

INTRODUCTION

Routine culturing of cells from solid non-small cell lung tumors would provide a valuable model for cytogenetic, biochemical, and molecular studies of bronchogenic carcinoma. Non-small cell solid tumors from the lung have been difficult to culture by standard techniques; success rates of 15% or less have been reported for different histological types using standard methods (1–12). Recent reports have achieved success rates of up to 45% using defined medium (13, 14). Our laboratory has established a technique for obtaining cultures from primary solid lung tumor tissue specimens with a high degree of success (91%), by using medium conditioned from an established bronchioloalveolar lung tumor cell line known to contain autocrine growth factors (15, 16). Parallel cultures of normal bronchial epithelial cells from the same donor were also established by explant culture (17) if a surgical specimen of the bronchus was available. These normal cells have been used as controls in histochemical procedures and assays of the neoplastic phenotype. The ability to examine biochemical and molecular characteristics of human lung tumor cells soon after culture and to compare their properties to replicating normal bronchial cells from the same patient may prove important in understanding the cellular changes occurring during the development of bronchogenic carcinoma.

MATERIALS AND METHODS

Cell Culture. The cell line A549-1 was selected from parent A549 cells, purchased from the American Type Culture Collection, by its ability to grow at low density (15). Maintenance of A549-1 and 3T3 cells were performed as described (15, 17). Normal bronchial epithelial cells were obtained from explant cultures in defined medium as described (17). Tumor cultures were established by mincing trimmed tumor tissue with two scalps and plating the cell mince onto Swiss 3T3 cells inactivated by γ-irradiation or by exposure to mitomycin C (15, 17). Suspension cultures were also attempted in some cases. A portion of tumor tissue and normal bronchus was fixed in formalin and stained with hematoxylin and eosin for a record of the original histology. Sections from the original tissue were also used as controls in immunohistochemical staining. Medium for primary tumor culture was a 1:1 mixture of Ham's F-12 (Gibco Laboratories, Grand Island, NY) and BME (Gibco) which had been conditioned for 48 to 72 h by A549-1 cells near confluence. This medium, termed CM medium, also contained 10% fetal bovine serum (Sterile Systems, Logan, UT). CM medium was prepared by first pelleting any cells present from the A549-1 cultures and filtering the conditioned medium twice through a 0.22-μm filter. The medium was also checked microscopically for the presence of any A549-1 cells. In experiments quantitating the growth stimulation of CM medium, a control medium, termed basal medium, was used for comparative purposes which consisted of a 1:1 mixture of Ham's F-12 and BME, supplemented with 10% fetal bovine serum. In some experiments serum was reduced to 1% or omitted.

Growth Assays. Colony assays in monolayer culture were performed on 3T3 feeder layers as described (15, 17). Soft agar assays were performed as described (15). Purified recombinant TGF-α was provided by Dr. Rik Derynck of Genentech, Inc., South San Francisco, CA. Purified IGF-1 was purchased from Collaborative Research, Bedford, MA.

Tumorigenicity Assay. Injections of cultured cells into female BALB/c mice were performed s.c. 0.4 to 2.0 × 106 cells were injected per site. The latency period for tumor formation was 3 to 6 mo. Tumors were fixed and stained for histological analysis and were also stained for expression of keratin, SP-1, and lung tumor antigen 47D10 as described below. Tumors arising from athymic mice were also analyzed for the presence of human LDH isoenzymes.

Immunohistochemistry. Antibodies for peroxidase-antiperoxidase visualization of antibody binding were purchased from Dako Laboratories, Santa Barbara, CA, or Vector Laboratories, Burlingame, CA. Keratin, SP-1, ACTH, and prolactin antibodies were rabbit anti-human IgGs. Antibody 47D10 was a mouse monoclonal prepared against A549 cell membranes and purchased from NEN-Dupont, Boston, MA; this antibody recognizes glycoproteins in adenocarcinomas of the lung but not normal lung. Control nonimmune rabbit serum was used to determine nonspecific background for rabbit antibodies; saline was used as a control for the mouse monoclonal antibody. Standard protocols prepared by the manufacturers were used for histochemistry. Cells from normal bronchial or tumor cultures were grown on slides to which 3T3 feeder cells had been attached. Colonies were allowed to form for 7 to 10 days, after which cells were fixed in methanol and used in staining protocols.

LDH Isoenzyme Profile. Cytosol was prepared from approximately 106 cells from cell pellets lysed by freeze-thawing in the presence of detergent. Cytosol was applied to agarose gels purchased from Intron.
Table 1. Primary cultures were established in 20 of 22 (91%) of different histologies using CM medium are presented in cultured by brief treatment with trypsin, which released some examined, some keratin expression was demonstrated. Ten of LDH Bands 1 to 5 was determined by densitometry, and the percentage of LDH A subunit present was calculated from the following formula

\[(LDH_3) + 0.75(LDH_4) + 0.5(LDH_5) + 0.25(LDH_2)\]

\(\times 100 = \% \text{ of A subunit}\)

RESULTS

Establishment of Cultures from Primary Lung Tumors. Results of attempts to culture cells from 22 cases of lung cancers of different histologies using CM medium are presented in Table 1. Primary cultures were established in 20 of 22 (91%). These cultures consisted of colonies of epithelial cells which formed on the 3T3 feeder cells within 1 to 2 wk (Fig. 1). Floating cells which did not attach were moved every 3 to 4 days to new flasks containing feeder cells. Cells placed in suspension culture with CM medium yielded viable cultures in 4 of 10 cases (1 case of large cell, 1 case of small cell, 1 case of squamous cell, and 1 case of adenocarcinoma). Of the 22 primary tumors placed in culture, 9 have yielded established cell lines which have been subcultured 5 to 20 times over a period of 8 mo to 2 yr.

The 3T3 feeder cells were beneficial for the attachment of primary tumor cells and for controlling fibroblast growth. Other substrates such as fibronectin and collagen (type IV) did not aid in attachment. This is in contrast to normal bronchial epithelial cells, which readily attach to either 3T3 cells or fibronectin-collagen substrates. Cells could most easily be subcultured by brief treatment with trypsin, which released some cells from established colonies. Feeder cells could be removed as desired after colonies became established by brief treatment with 0.002% EDTA solution, thereby producing purified populations of lung tumor cells.

Demonstration of Neoplastic Phenotypes of Cultured Lung Tumors. In 12 of the 20 cases cultured, a series of assays were performed to verify the expression of neoplastic phenotypes. These results are summarized in Table 2. In all tumor cultures examined, some keratin expression was demonstrated. Ten of 11 cultures examined also expressed the SP-1 glycoprotein. In a few cases some ACTH or prolactin was expressed by the tumor cultures. Cultures derived from 5 of the 9 cases examined have produced tumors in nude mice to date; these tumors were shown to originate from the neoplastic lung cultures by at least one of these criteria: histologic features; expression of human LDH isoenzymes; expression of keratin; expression of SP-1 protein; or expression of a lung tumor antigen recognized by antibody 47D10 (data not shown). Normal bronchial epithelial cells cultured from the same donors were used as controls in immunochemical staining. Normal bronchial cells did express keratin, but did not express any SP-1 protein, ACTH, or prolactin. Normal cells also did not form tumors in athymic mice (Table 2).

Anchorage-independent growth was also used to assess the phenotype of cells cultured from lung tumors (Table 2). In 8 of 8 cases, tumor cells were able to form colonies in soft agar, at a cloning efficiency of from 17.3 to 4053.4 foci per 10^5 cells seeded in medium without conditioned medium added. Normal bronchial epithelial cells did not form colonies in soft agar.

Altered expression of LDH isoenzymes was also used as a criterion for neoplastic phenotype (Fig. 2). Compared to normal epithelial cells cultured from bronchus, most tumor cultures expressed more of the muscle-type LDH isozymes (LDH 4 and 5) and less of the heart-type isozymes (LDH 1 and 2), reflecting an increase in gene products from the LDH A gene. This was not true of every tumor and may reflect specific alterations in chromosome 11 occurring in individual cases. The difference in isozyme pattern was quantitated by determining the percentage of total LDH represented by LDH A as described in “Materials and Methods.” For normal cells, this was 23.4 to 28.3%; in most tumors it was increased: 81-86T, 60.0%; 101-87T, 64.9%; 105-87T, 73.8%; 109-87T, 51.3%; 110-87T, 48.1%; and 114-87T, 37.4%. In one case the percentage of LDH was not altered (91-86T, 25.8%).

Stimulation of Tumor Cell Growth by CM Medium. Colony formation of tumor cells in both monolayer culture (Table 3) and soft agar (Table 4) was stimulated by CM medium. In all cases tested, cells were first cultured in CM for at least 2 wk in order to obtain primary colonies of purified tumor cells. This medium was then washed away during harvesting of the cells, which were resuspended in either basal medium or CM medium. The original primary tumor tissue was not used for colony assays for 2 reasons: (a) enzymatic disruption of tissue to yield single-cell suspensions was found to reduce viability considerably; and (b) such suspensions would be a mixture of connective tissue and tumor cells, and the autocrine factors known to be present in CM medium could also stimulate any fibroblasts present. Without initial culturing in CM, very low yields of primary cells were obtained. We therefore have not examined the effects of CM medium on “naive” tumor cells. A 1.7- to 1.9-fold increase in colony-forming efficiency was observed in CM medium compared to basal medium in cultures at passage 1 or higher (Table 3). In the case of 105-87T, a large cell carcinoma, primary cells showed a 6.6-fold stimulation by CM medium, which was reduced to 1.7-fold by passage 2. The extent of stimulation of anchorage-independent growth by CM medium varied by 2.0- to 7.9-fold in different tumor cell cultures (Table 4).

Effects of Purified Growth Factors on Cultured Tumor Cells. Conditioned medium from A549-1 cells has previously been shown to contain TGF-α and IGF-1-like peptides (15, 16). The response of newly cultured squamous lung carcinoma cells (specimen 101-87T) to these purified growth factors was examined in the range of concentrations expected in CM medium and at higher levels (Fig. 3). Maximum stimulation of colony formation by TGF-α was observed at 0.1 ng/ml, and at 10 ng/ml, inhibition of colony formation was observed. Maximal stimulation by IGF-1 was observed at 10 ng/ml. These results were obtained using 1% fetal bovine serum. The combined effect of TGF-α and IGF-1 was examined in a colony-forming assay in the absence of serum, using cells which had been grown for 2 wk in 1% fetal bovine serum (Fig. 4). This was done to

Table 1 Results of culturing human solid lung tumors

<table>
<thead>
<tr>
<th>Tumor histology</th>
<th>Primary cultures</th>
<th>Subcultures</th>
<th>Cell line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Squamous cell</td>
<td>7/8</td>
<td>7/8</td>
<td>3/8</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>4/5</td>
<td>3/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Adenosquamous</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Large cell</td>
<td>5/5</td>
<td>4/5</td>
<td>3/5</td>
</tr>
<tr>
<td>Small cell</td>
<td>1/1</td>
<td>1/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Total</td>
<td>20/22</td>
<td>18/22</td>
<td>9/22</td>
</tr>
</tbody>
</table>
Fig. 1. Phase-contrast photomicrographs of cultured lung tumor cells. A, 91-86T, passage 5, cultured without feeder cells, x 900. B, 101-87T, passage 1, cultured with feeder cells, x 2250. C, 102-87T, primary culture, with feeder cells, x 2250.

Table 2 Demonstration of neoplastic and epithelial phenotype in newly cultured lung carcinomas

<table>
<thead>
<tr>
<th>Tumor specimen</th>
<th>Histology</th>
<th>Expression of keratin</th>
<th>Expression of SP-1</th>
<th>Expression of ACTH</th>
<th>Expression of PRL</th>
<th>Tumorigenicity in immunodeficient mice</th>
<th>Cloning efficiency in soft agar (foci/10^4 cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>81-86T</td>
<td>Large cell</td>
<td>±</td>
<td>−</td>
<td>±</td>
<td>±</td>
<td>Positive</td>
<td>93.3</td>
</tr>
<tr>
<td>91-86T</td>
<td>Adenosquamous carcinoma</td>
<td>+++</td>
<td>++</td>
<td>+</td>
<td>−</td>
<td>Positive</td>
<td>126.7</td>
</tr>
<tr>
<td>101-87T</td>
<td>Squamous</td>
<td>+++</td>
<td>+++</td>
<td>±</td>
<td>±</td>
<td>Positive</td>
<td>44.3</td>
</tr>
<tr>
<td>102-87T</td>
<td>Large cell</td>
<td>ND*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Positive</td>
<td>48.3</td>
</tr>
<tr>
<td>105-87T</td>
<td>Large cell</td>
<td>+++</td>
<td>+++</td>
<td>−</td>
<td>−</td>
<td>Positive</td>
<td>4053.4</td>
</tr>
<tr>
<td>108-87T</td>
<td>Adenocarcinoma</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>Negative</td>
<td>17.3</td>
</tr>
<tr>
<td>109-87T</td>
<td>Squamous</td>
<td>+++</td>
<td>+++</td>
<td>−</td>
<td>±</td>
<td>In progress</td>
<td>60.6</td>
</tr>
<tr>
<td>110-87T</td>
<td>Adenosquamous carcinoma</td>
<td>+++</td>
<td>+++</td>
<td>−</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>113-87T</td>
<td>Large cell</td>
<td>++</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>114-87T</td>
<td>Squamous</td>
<td>++</td>
<td>+</td>
<td>±</td>
<td>−</td>
<td>ND</td>
<td>20.0</td>
</tr>
<tr>
<td>115-87T</td>
<td>Adenocarcinoma</td>
<td>++</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>Negative</td>
<td>ND</td>
</tr>
<tr>
<td>116-87T</td>
<td>Squamous</td>
<td>+++</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Negative</td>
<td>0.0</td>
</tr>
<tr>
<td>Normal bronchial epithelial cells</td>
<td>+++</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>Negative</td>
<td></td>
</tr>
</tbody>
</table>

ND, not determined.
try to create conditions in which the tumor cells would be maximally responsive to added growth factors. The colony-forming efficiency was much reduced without serum, but a stimulation by the 2 growth factors was apparent at 0.1 and 0.5 ng/ml of TGF-α and at levels of IGF-1 from 0.01 to 10 ng/ml. An additive effect of TGF-α and IGF-1 was observed at concentrations of TGF-α of 0.2 to 0.5 ng/ml combined with IGF-1 at concentrations of 0.1 to 1.0 ng/ml. (Conditions J, K, M, and N in Fig. 4). This demonstrates that low levels of growth factors in combination (such as have been detected in CM medium) are very effective in promoting cell growth.

**Table 3** Conditioned medium stimulation of colony formation of lung tumor cultures in monolayer culture

<table>
<thead>
<tr>
<th>Tumor culture</th>
<th>No. of cells seeded/well</th>
<th>Basal medium (No. of colonies/well)</th>
<th>CM medium (No. of colonies/well)</th>
<th>Fold stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>91-86T, passage 3</td>
<td>5000</td>
<td>332.0 ± 45.4</td>
<td>631.3 ± 25.1</td>
<td>1.9</td>
</tr>
<tr>
<td>101-87T, passage 2</td>
<td>5000</td>
<td>213.0 ± 47.3</td>
<td>409.7 ± 109.2</td>
<td>1.9</td>
</tr>
<tr>
<td>105-87T, passage 0</td>
<td>5000</td>
<td>76.0 ± 30.0</td>
<td>504.3 ± 120.1</td>
<td>6.6</td>
</tr>
<tr>
<td>105-87T, passage 2</td>
<td>2000</td>
<td>125.0 ± 13.1</td>
<td>212.0 ± 13.7</td>
<td>1.7</td>
</tr>
<tr>
<td>109-87T, passage 1</td>
<td>2000</td>
<td>11.0 ± 2.2</td>
<td>21.3 ± 6.2</td>
<td>1.9</td>
</tr>
</tbody>
</table>

* Mean ± SEM.  
** P < 0.01, two-tailed t test.  
* P < 0.09, two-tailed t test.  
* P < 0.05, two-tailed t test.

**Table 4** Conditioned medium stimulates anchorage-independent growth of primary lung tumor cells

<table>
<thead>
<tr>
<th>Tumor culture</th>
<th>No. of cells seeded/well</th>
<th>Basal medium (No. of colonies/well)</th>
<th>CM medium (No. of colonies/well)</th>
<th>Fold stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>81-86T, passage 18</td>
<td>1.0 x 10^5</td>
<td>93.3 ± 21.1</td>
<td>509.3 ± 160.5</td>
<td>5.5</td>
</tr>
<tr>
<td>91-86T, passage 4</td>
<td>0.3 x 10^5</td>
<td>38.0 ± 4.3</td>
<td>106.3 ± 26.1</td>
<td>2.8</td>
</tr>
<tr>
<td>101-87T, passage 0</td>
<td>0.4 x 10^5</td>
<td>17.7 ± 8.5</td>
<td>35.3 ± 8.1</td>
<td>2.0</td>
</tr>
<tr>
<td>102-87T, passage 0</td>
<td>0.4 x 10^5</td>
<td>19.3 ± 8.6</td>
<td>38.3 ± 11.9</td>
<td>2.0</td>
</tr>
<tr>
<td>105-87T, passage 2</td>
<td>0.5 x 10^5</td>
<td>202.6 ± 294.5</td>
<td>4360.0 ± 981.4</td>
<td>2.2</td>
</tr>
<tr>
<td>106-87T, passage 1</td>
<td>0.5 x 10^5</td>
<td>3.0 ± 2.2</td>
<td>23.7 ± 6.3</td>
<td>7.9</td>
</tr>
<tr>
<td>109-87T, passage 1</td>
<td>0.1 x 10^5</td>
<td>6.0 ± 2.2</td>
<td>42.3 ± 18.9</td>
<td>7.1</td>
</tr>
<tr>
<td>115-87T, passage 0</td>
<td>0.1 x 10^5</td>
<td>2.0 ± 1.4</td>
<td>11.3 ± 4.0</td>
<td>5.7</td>
</tr>
</tbody>
</table>

* Mean ± SEM.  
** P < 0.02, two-tailed t test.  
* P < 0.01, two-tailed t test.  
* P < 0.09, two-tailed t test.  
* P < 0.05, two-tailed t test.  
/ P < 0.06, two-tailed t test.  

**DISCUSSION**

A highly successful method for culture of purified tumor cells from human solid lung tumors has been established. Condi-
tioned medium used as a growth supplement has previously been shown (16) to contain at least 2 factors which can stimulate cell growth: TGF-α (19–21) and IGF-1 (Refs. 22 and 23; Footnote 4). Recent work has shown that IGF-1 is a mitogen for small cell lung carcinoma cells (Ref. 23; Footnote 4). The non-small cell tumors cultured using this medium yielded malignant cells based on the expression of a variety of tumor phenotypes and are clearly different from normal epithelial cells cultured from the bronchus of the same tissue donors, or from other donors. Mutually exclusive growth conditions (serum-containing conditioned medium for tumors, defined medium for normals) were used to ensure culture of distinct cell types. The use of a feeder layer facilitated the attachment and growth of primary tumor cells, and in some cases tumor cells became independent of feeder layers with time in culture. Klein et al. (24) have also described the use of murine fibroblasts (10T½ cells) for the primary culture of bronchial carcinomas. The effect of 3T3 cells could be due in part to factors secreted by the cells, rather than merely one of attachment. In tests of 3T3 cell conditioned medium, however, only a slight (less than 20%) stimulation of colony formation of primary tumor cells was found. Attempts to use A549-1 cells as the feeder layer were not successful; neither bronchial epithelial cells nor primary tumor cells would attach to an irradiated layer of A549-1 cells. Mesenchymal cells may therefore provide a better milieu for attachment.

Lung tumor cultures expressed two known markers of lung carcinomas: production of immunoreactive SP-1 protein (25, 26) and alterations in LDH isoenzyme profiles (27). Some ACTH and prolactin, which also are expressed ectopically by some bronchogenic carcinomas (28, 29), were also observed. The fact that these proteins were observed in tumor sections but not in sections or cultures of normal bronchial epithelial cells suggests that the markers are tumor specific and not related to artifacts arising from cell culture.

Clonal assays were used to demonstrate the stimulatory effect of conditioned medium on tumor cells. For comparison, the purified growth factors TGF-α and IGF-1 were added in clonal assays in the range of concentrations which would be expected to be present in conditioned medium. The estimated secretion of TGF-α by A549-1 cells is 0.25 to 0.50 ng/ml (15, 16), and the estimated secretion of IGF-1 is 0.1 ng/ml. A combination of these 2 factors at these levels produced growth stimulation as measured in a colony-forming assay using cells cultured from a solid squamous cell carcinoma. This suggests that the growth stimulation observed with conditioned medium is caused at least in part by these 2 factors, although other unknown factors cannot be ruled out. A recent report indicates that a M, 20,000 autocrine peptide exists which specifically stimulates cell growth: TGF-α (19-21) and IGF-1 (Refs. 22 and 23; Footnote 4). The observation that high levels of TGF-α (10 ng/ml) produced a decrease in colony formation suggests that TGF-α, like EGF, can induce differentiation as well as growth stimulation, under the proper conditions.

Primary tumor cells may be most responsive to added growth factors immediately after being placed in culture. After being established in culture, the cells may be stimulated to produce the autocrine factors themselves. Coffey et al. have recently demonstrated that, in keratinocytes, the synthesis of TGF-α messenger RNA and protein is induced by addition of EGF or TGF-α (32). Our observation that the extent of growth stimulation declined with time in culture with the autocrine factors may reflect the same phenomenon. It is possible that the same factors which stimulate cell proliferation eventually contribute to cell differentiation and might explain why subcultures of primary tumor cells are obtained at lesser frequency than primary cultures.

ACKNOWLEDGMENTS

The authors thank Dr. George Michalopoulos and Alan Novotny of Duke University for obtaining human specimens and donor information and for performing injections into athymic mice, and Dr. Rik Derynck of Genentech, Inc., for providing recombinant TGF-α.

REFERENCES

16. Siegfried, J. M., and Neasnow, S. Cytotoxicity of chemical carcinogens to-

Response of Primary Human Lung Carcinomas to Autocrine Growth Factors Produced by a Lung Carcinoma Cell Line

Jill M. Siegfried and Sara E. Owens


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/48/17/4976

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.