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Culture of Primary Lung Tumors Using Medium Conditioned by a Lung Carcinoma Cell Line

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Medium conditioned by the established lung tumor cell line A549 was used as a supplement to culture cells from primary solid lung tumors. Of 36 cases placed into culture, primary cells were obtained in 33 (91.7%). Of 29 cases in which subcultures were attempted, 18 (62.1%) were successful. Nine cell lines have been established by this technique to date. In growth assays, conditioned medium (CM) was found to stimulate both monolayer colony formation and growth in semi-solid medium of cells cultured from primary solid tumors. CM has been found to contain factors with the properties of both transforming growth factorα (TGFα) and insulin-like growth factor-I (IGF-I). The addition of a combination of these factors as purified peptides to basal medium at levels found in CM (0.1–0.5 ng/ml) stimulated colony formation of lung tumor cells by up to fourfold. These results indicate that secretion of growth factors may be important in tumor growth in vivo, and that use of CM may be a valuable tool for obtaining cultures from primary solid tumors.

Key words: lung cancer, TGFα, IGF-I

Evidence from different human and animal studies indicates that many tumor types are capable of producing and secreting growth factors [1–3]. Definitive proof that production of these factors is necessary for the neoplastic state has not been demonstrated, but circumstantial evidence indicates that these autocrine factors can promote growth of both tumor and normal cells. CM from the lung carcinoma cell line A549, containing such factors [4–6], was used to stimulate cells from primary solid non-small-cell lung tumors to divide in culture. Non-small-cell lung tumors have been very difficult to grow in culture by standard techniques, in contrast to small cell carcinoma, which has been cultured with more success [7,8]. The ability to obtain cultures routinely represents an important advance in ability to study the cytogenetics, molecular, and cell biology of non-small-cell carcinoma, which represents 80% of lung tumors.

Abbreviations used: CM, conditioned medium; TGFα, transforming growth factorα; IGF-I, insulin-like growth factor-I.

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MATERIALS AND METHODS

Cell Culture

Production of CM from A549 cells [4] and culture and inactivation of Swiss 3T3 cells [5] have been described previously. Tumor cultures were established by mincing tissue with two scalpels and plating released cells and fragments onto flasks of inactivated Swiss 3T3 cells [4,5]. Medium for tumor culture was a 1:1 mixture of fresh Ham's F12 and Basal Medium Eagle's which had been conditioned for 48 hr by A549 cells, obtained from the American Type Culture Collection. Both Ham's F12 and CM contained 1% fetal bovine serum. CM was centrifuged to remove any A549 cells present and filtered under positive pressure through a 0.22 μm filter before being used as a medium supplement.

Growth Assays

Colony assays on 3T3 feeder layers and growth in semi-solid medium were performed as described [4,5,9]. Basal medium used as a control consisted of a 1:1 mixture of fresh Ham's F12 and fresh Basal Medium Eagle's which had not been conditioned. Recombinant IGF-I was obtained from IMCERA Bioproducts, Inc. (Terre Haute, IN). Recombinant TGFα was obtained from Dr. Rik Derynck, (Genentech, Inc.).

RESULTS AND DISCUSSION

Table I summarizes results of culturing primary solid lung tumors of various histologies using A549 CM. In 91.7% of cases, primary cultures were established. These cultures consisted of colonies of carcinoma cells which formed on 3T3 feeder layers in 1–2 weeks, and could easily be distinguished from the fibroblast feeder layer (Fig. 1). These cultures were confirmed to be of neoplastic origin by expression of tumor markers and growth in immunosuppressed mice [5], and provided sufficient mitotic cells for karyotypic analysis and assay of growth requirements. Subcultures were obtained in 62.1% of cases attempted, and in nine cases a cell line was established. This success rate may make it possible to perform routine karyotypic and molecular analysis of proliferating cells from solid non-small-cell lung tumors. 3T3 feeder cells appear to function in promoting attachment of tumor cells to the flasks; CM from 3T3 cells had little ability to stimulate growth of colonies [4].

TABLE I. Culture of Primary Solid Lung Tumors

Tumor histology	Primary culture	Subcultures
Squamous cell	12/13	6/10
Adenocarcinoma	4/6	2/5
Adenosquamous	5/5	4/4
Large cell	6/6	4/6
Small cell	2/2	0/2
Undetermined[a]	4/4	2/2
Total	33/36	18/29

[a]Pathology reports incomplete to date.

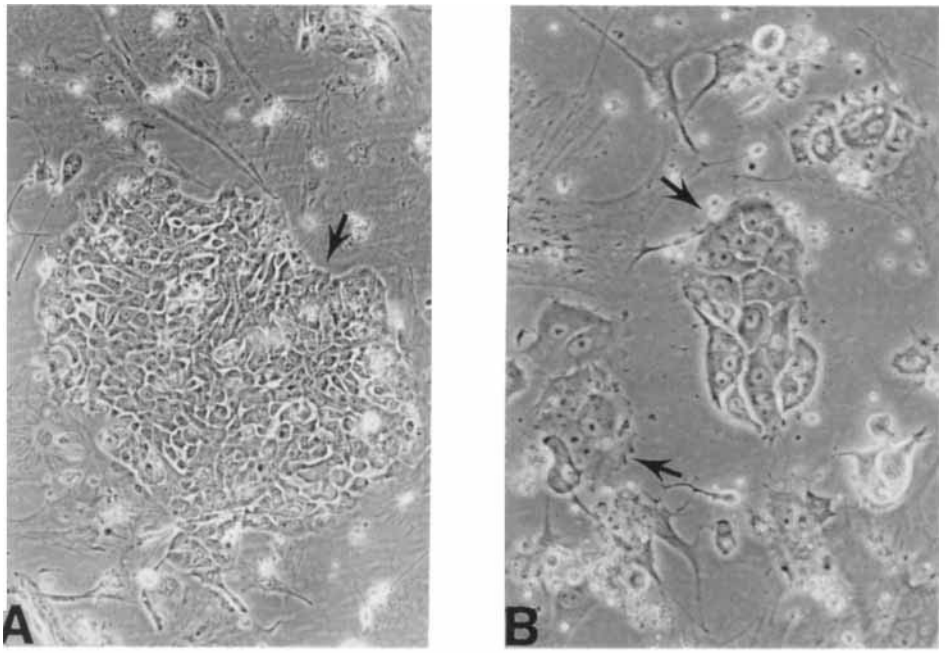

Fig. 1. Phase-contrast photomicrograph of primary solid tumor cells growing on 3T3 feeder layers. **A:** Adenosquamous carcinoma. ×350. **B:** Adenocarcinoma. ×900. Arrows indicate colonies of carcinoma cells which form on feeder layer.

TABLE II. Colony Formation of Cultured Lung Tumor Cells in Monolayer Culture*

Condition	Colony-forming efficiency
Basal medium	6.64 ± 0.9
25% CM	12.1 ± 0.4
50% CM	12.6 ± 0.5

*Results from specimen 91-86 (adenosquamous cancer), 5,000 cells seeded per well

CM was shown to stimulate cell proliferation of primary tumor cells in quantitative clonal assays. In both monolayer culture (Table II) and semi-solid suspension culture (Table III), CM increased colony-forming efficiency 2.0–7.1-fold compared to basal medium. Colonies observed in the presence of CM also were larger as a group than those seen in basal medium. These data confirm that CM is effective in inducing cell proliferation in primary tumor cells.

CM from A549 cells has been shown to contain both TGFα- and IGF-I-like peptides at levels of 0.1–0.5 ng/ml [4,6]. Purified recombinant peptides were used in a clonal assay to demonstrate responsiveness of primary lung tumor cells to peptides at the levels detected in CM (Fig. 2). This assay was performed in serum-free medium in order to maximize response to the peptides. Combining low levels of the two peptides produced a 3–4-fold greater colony-forming efficiency than the control condition (Fig. 2). This effect was more than additive for that of the two peptides alone. This result indicates that

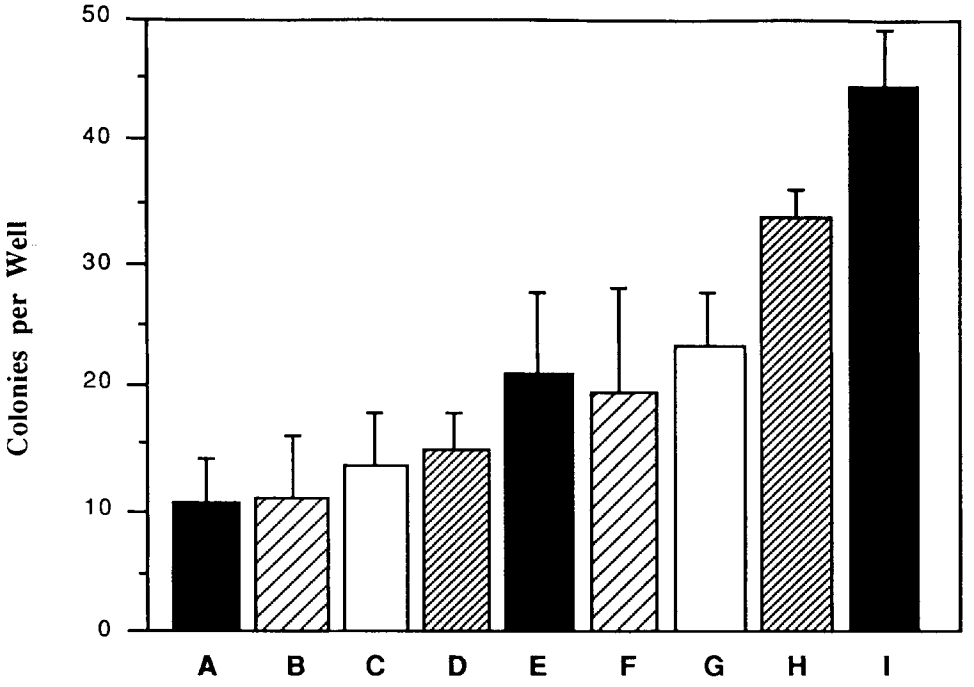

Fig. 2. Effect of purified growth factors TGFα and IGF-I alone and in combination on colony formation of cells derived from specimen 101-87, squamous cell carcinoma of lung. **A:** Control. **B:** TGFα, 0.05 ng/ml. **C:** TGFα, 0.2 ng/ml. **D:** TGFα, 0.5 ng/ml. **E:** IGF-I, 0.01 ng/ml. **F:** IGF-I, 0.1 ng/ml. **G:** IGF-I, 1.0 ng/ml. **H:** TGFα, 0.2 ng/ml plus IGF-I, 0.1 ng/ml. **I:** TGFα, 0.5 ng/ml plus IGF-I, 0.1 ng/ml. Two thousand cells were plated per well containing inactivated 3T3 cells in serum-free basal medium. Cells were fixed and stained after 7 days.

TABLE III. Anchorage-Independent Growth of Primary Lung Tumor Cells

		Cloning efficiency[a]	
Specimen	Histology	Basal medium	CM
101-87	Squamous cell	4.4 ± 2.1	8.8 ± 2.0
102-87	Large cell	4.8 ± 2.2	9.6 ± 2.9
109-87	Squamous cell	6.0 ± 2.2	42.3 ± 18.9
115-87	Adenocarcinoma	2.0 ± 1.4	11.3 ± 4.0

[a]Colonies formed per 10^4 cells seeded. Cultures were assayed as primary cultures or at passage 1.

low levels of secreted growth factors acting in consort are very effective in promoting growth of primary lung tumor cells. Presence of CM from A549 cells may act to substitute temporarily for factors tumor cells are capable of producing; such factors may not be produced initially during the transition from in vivo to in vitro growth, contributing to the difficulty in culturing cells from primary tumors without supplementation with CM.

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