Heterogeneity in the Production of Collagens and Fibronectin by Morphologically Distinct Clones of a Human Tumor Cell Line: Evidence for Intradural Diversity in Matrix Protein Biosynthesis

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INTRODUCTION

Genetic and phenotypic diversity exists in most, if not all, solid tumors (1–3). Neoplastic cells derived from a single tumor may show heterogeneity with respect to morphology, genome size, organization, growth rate, metabolic activity, antigenic expression, clonogenicity, and invasiveness in vitro (e.g., 4–8). Recent studies have also provided evidence for heterogeneity in the expression of matrix protein receptors (e.g., for laminin and fibronectin) and in the secretion of matrix-degrading enzymes, such as type IV collagenase (9–11). Thus, intratumoral diversity may be essential to a tumor's ability to grow, invade, and metastasize and may ultimately determine its responsiveness to therapeutic intervention.

We recently described the isolation and characterization of a polyclonal human lung tumor cell line, designated JH-17. The tumor was isolated from the pleural effusion of a patient with a widely disseminated pulmonary malignancy, clinically diagnosed as a large cell undifferentiated carcinoma of the lung. For the present studies, freshly thawed cells were seeded and propagated in Dulbecco's modified Eagle's medium supplemented with 25 mM Hepes, pH 7.5, and 10% fetal calf serum (Hazelton).

MATERIALS AND METHODS

Cell Culture. Cells were isolated from pleural fluid and propagated as described by Stone and associates. JH-17 was cloned by limiting dilution at transfer 7 (1:3 split ratio), and initial biochemical characterization of individual clones was performed at transfers 4 and 5. Most protein isolation studies were performed using cells at transfers 7 to 12. Flow cytometric analysis of DNA content, karyotyping, Mycoplasma screening, and studies of growth kinetics, clonogenicity, and tumorigenicity were performed as described by Stone et al. For the present studies freshly thawed cells were seeded and propagated in Dulbecco's modified Eagle's medium (Washington University Cell Culture Facility, St. Louis, MO) supplemented with 25 mM Hepes, pH 7.5, and 10% fetal calf serum (Hazelton).

Metabolic Labeling of Cells. Nearly confluent cultures were washed with medium and preincubated for 30 min with serum-free Dulbecco's modified Eagle's medium supplemented with 25 mM Hepes, pH 7.5, 50 µg/ml fresh sodium ascorbate, and 80 µg/ml l-aminopropionitrile fumarate. Cultures were labeled for 24 h with medium containing 20 µCi/ml of [2-3H]proline (New England Nuclear, 40 Ci/mmol).

Processing of Culture Medium and Attached Cells. The culture medium and one TBS2 wash of the culture dish were harvested into inhibitors (0.2 mM PMSF, 2 mM N-ethylmaleimide, 2.5 mM EDTA, and 100 µg/ml l-aminopropionitrile fumarate at 4°C; the plates were washed twice with TBS containing inhibitors and temporarily stored at −20°C. All subsequent procedures were performed at 4°C except as indicated. The medium was clarified by centrifugation at 400 x g for 15 min to remove cellular debris, and the supernatant was further clarified by centrifugation for 30 min at 48,000 x g for SDS-PAGE, the medium was dialyzed versus 0.1 M acetic acid in the presence of pepstatin (Sigma, 1 µg/ml) and lyophilized. Attached cells and matrix samples were processed as described above. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
were scraped into 0.1 M acetic acid containing pepstatin, briefly homogenized with a Polytron (Brinkmann), dialyzed against acetic acid, and lyophilized.

**Quantification of Newly Synthesized Proteins.** Radiolabeled proteins in aliquots of clarified medium or cell layer homogenates were precipitated with 10% (w/v) TCA at 0-4°C, resolubilized in 1 N NaOH, neutralized, and quantified by liquid scintillation spectrometry. The proportion of radioactivity in collagenous protein was quantified by bacterial collagenase assay and radioactive hydroxyproline and proline determinations as previously described (12).

**SDS-PAGE and Fluorescence Autoradiography.** Proteins were resolved by SDS-PAGE on discontinuous methylenebis(acrylamide) slab gels containing 0.5 M freshly deionized urea and visualized by fluorescence autoradiography (13). The relative recovery of radioactivity in proteins resolved by SDS-PAGE was determined by densitometry of appropriately exposed fluorograms (14). Molecular weights were determined relative to internal type I collagen α chains, dimers, and trimers.

**2-Dimensional SDS-PAGE.** Patterns of interchain disulfide bonding were examined by 2-dimensional SDS-PAGE essentially as described by Altalal et al. (15). Briefly, unredyed proteins were resolved by SDS-PAGE on 0.75-mm-thick 3%/5% slab gels. The first dimension gel strips were incubated for 10 min at 37°C in sample buffer containing 50 mM DTT, horizontally positioned above a 1.5-mm-thick 3%/6% slab gel, and sealed in place with agarose containing 50 mM DTT. Reduced proteins were resolved by SDS-PAGE in the second dimension.

**Purification of Newly Synthesized Collagens.** Collagens were precipitated from clarified media with 30% (w/v) ammonium sulfate at 4°C and further purified by ion exchange chromatography on a column of DE-52 cellulose (Whatman, 1.2 x 5 cm) equilibrated at 4°C with 6 M urea, 75 mM NaCl, 50 mM Tris-HCl, pH 7.5, 2.5 mM EDTA, 0.2 mM PMSF (16). After the initial wash, bound proteins were eluted with a linear gradient of 75-375 mM NaCl over a total volume of 200 ml at a flow rate of 60 ml/h; 3-ml fractions were collected. Conductivities were measured at 4°C. Recoveries were routinely greater than 80%. In some cases, proteins eluted from DE-52 were further purified by chromatography on a column of CM-52 cellulose (1.2 x 5 cm) equilibrated at 42°C with 6 M urea, 0.04 M sodium acetate, pH 4.8, and eluted with a linear gradient of 0-0.2 M NaCl over a total volume of 200 ml (17). Recoveries were greater than 70%. Molecular sieve chromatography was performed using a column of 6% agarose (Bio-Rad; Bio-Gel A-5M; 200-400 mesh, 1.5 x 165 cm) equilibrated at room temperature with 2 M GuHCl, 50 mM Tris-HCl, pH 7.5 (18). Pepstatin was added to pooled column fractions which were then dialyzed against 0.1 M acetic acid, lyophilized, and examined by SDS-PAGE.

**Proteolytic and Chemical Cleavage of Proteins.** Lyophilized proteins were digested with pepsin (Worthington; 3352 units/mg) in 0.5 M acetic acid (50 μg/ml) for 24 h at 4°C. Digestion of TCA-insoluble or lyophilized proteins with purified bacterial collagenase was performed as previously described (12). Although the collagenase (Form III; Advance Biofactores; 67 units/μg) showed no detectable proteolytic activity against radiolabeled noncollagenous substrates, digests were routinely performed in the presence of 0.2 mM PMSF and 2 mM N-ethylmaleimide. CNBr peptide mapping of proteins in gel slices was performed by the method of Barsh et al. (19).

**Immunoblotting.** Monospecific antibodies to human placenta type IV collagen and human amnion type V collagen were prepared in rabbits. The type IV antibodies were affinity purified and characterized as previously described (13). Monospecific polyclonal antibodies to human plasma fibronectin were kindly provided by Dr. John McDonald (Department of Medicine, Washington University School of Medicine, St. Louis, MO). Rabbit antibodies to bovine type VIII collagen and human placenta type V collagen were kindly provided by Drs. Raj Kapoor and Helen Sage (Department of Biological Structure, University of Washington, Seattle, WA). Immunoprecipitations of dialyzed culture medium were performed as previously described (18), except that immune complexes were precipitated with goat anti-rabbit IgG. Immunoblotting was performed by the method of Towbin et al. (20); electrophoretic transfers were performed in the absence of methanol.

**Amino Acid Analysis.** Lyophilized proteins were dissolved in constant boiling HCl, flushed with nitrogen, and hydrolyzed in vacuo at 108°C for 24 h. Analyses were performed on a Beckman 119C amino acid analyzer modified for single-column microanalysis. The positions of elution of 4-hydroxyproline and hydroxylysine were determined by prior calibration with amino acid standards.

**RESULTS**

Early passage cultures of the parental JH-17 cell line have been extensively characterized. The initial pleural fluid isolates and early passage cultures were morphologically heterogeneous by microscopy and demonstrated at least two discrete aneuploid populations by flow cytometry. JH-17 was clonogenic in soft agar and readily formed tumors in athymic nude mice. Immunochemistry. Monospecific antibodies to human placenta type IV collagen and human amnion type V collagen were prepared in rabbits. The type IV antibodies were affinity purified and characterized as previously described (13). Monospecific polyclonal antibodies to human plasma fibronectin were kindly provided by Dr. John McDonald (Department of Medicine, Washington University School of Medicine, St. Louis, MO). Rabbit antibodies to bovine type VIII collagen and human placenta type V collagen were kindly provided by Drs. Raj Kapoor and Helen Sage (Department of Biological Structure, University of Washington, Seattle, WA). Immunoprecipitations of dialyzed culture medium were performed as previously described (18), except that immune complexes were precipitated with goat anti-rabbit IgG. Immunoblotting was performed by the method of Towbin et al. (20); electrophoretic transfers were performed in the absence of methanol.

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Preliminary biochemical characterization of JH-17 indicated that approximately 30% of the TCA-insoluble [3H]proline-labeled protein in the culture medium was in bacterial collagenase-sensitive sequences. SDS-PAGE of the unfraccionated culture medium following a 24-h labeling period revealed several high M, bacterial collagenase-sensitive components, including species tentatively identified as proα1(V), proα2(V), proα2(IV), proα2(IV), and type VIII collagen. Similar analysis of proteins secreted by various clones derived from JH-17 revealed considerable heterogeneity in the pattern of high M, proline-labeled components (see below). The further characterization of several of these clones is described below.

Characterization of Clone 13. Clone 13 (C13) was comprised of pleomorphic cells with numerous short cytoplasmic processes (Fig. 1, left), which appeared somewhat polygonal in confluent culture. Ultrastructural studies demonstrated abundant cytoplasmic microfilaments, occasional junctional complexes, absence of a defined basal lamina, and a microfilamentous extracellular matrix. The cells had a DNA index of 1.4 and a population doubling time of approximately 32 h. Although the DNA index and growth parameters were similar to those of the parental JH-17 culture, C13 was not observed to grow in soft agar or to form tumors in nude mice.

The relative collagen synthetic activity of C13 was similar to that of the parent JH-17 culture. Approximately 40% of the TCA-insoluble [3H]proline in the culture medium was in bacterial collagenase-sensitive sequences (40 ± 2%, mean ± SE for three separate sets of cultures). Although collagenase-sensitive components accounted for approximately 1% (1.0 ± 0.09%) of the TCA-insoluble radioactivity in the cell layer, nearly 80% of the collagenous protein was recovered in the culture medium. Comparable results were obtained using radioactive hydroxyproline and proline assays suggesting that the secreted collagenous proteins had relatively normal levels of prolyl hydroxylation.

SDS-PAGE of nondialyzable culture medium proteins revealed at least five bacterial collagenase-sensitive components (Fig. 2, upper) that comigrated with the major collagenous components elaborated by the parent cell line. The collagenous proteins were concentrated by precipitation with ammonium sulfate and further characterized by SDS-PAGE in the presence or absence of sulfhydryl reduction, ion exchange chromatography, chemical and enzymatic peptide mapping, and immunoblotting. Four of the bacterial collagenase-sensitive chains secreted by C13 cells were identified as type V chains and processing intermediates based on the following observations. (a) The chains cross-reacted in immunoprecipitation assays with polyclonal antibodies to native human placenta type V collagen (supplied by Dr. Helene Sage) and with antibodies to denatured human amnion type V collagen on immunoblots (Fig. 3). (b) Limited pepsin digestion yielded two major pepsin-resistant fragments that comigrated with authentic type V collagen chains on SDS-PAGE (Fig. 4, first dimension gel, left panel) and eluted with pepsinized type V chains from CM-cellulose (not shown). The ratio of α1(V) to α2(V) for the radiolabeled collagen was approximately 2.1 consistent with collagen trimers of the composition [α1(V)]2α2(V). (c) Two-dimensional SDS-PAGE and CNBr mapping confirmed the relationship of the putative proα(V), proα(V), and proα(V) dimers to type V collagen (Figs. 2B and 4). CNBr peptide mapping of the silver-stained pepsin-resistant components also demonstrated extensive homology with peptides derived from human amnion type V collagen on SDS-PAGE (not shown). (d) Hydroxyproline: proline and hydroxylysine:lysine ratios obtained by amino acid analysis of proteins secreted by various clones derived from JH-17 revealed considerable heterogeneity in the pattern of high M, proline-labeled components (see below). The further characterization of several of these clones is described below.

Fig. 2. Synthesis and secretion of proteins by C13. Upper, cultures of C13 cells were labeled for 24 h with [3H]proline. Collagens were concentrated with 30% (w/v) ammonium sulfate, resolved by SDS-PAGE with or without prior reduction with DTT, and visualized by fluorography. Lane 1, reduced. Lane 2, unreduced. Bands were identified as described in the Results section and subsequent figures. Left, positions of reduced proα1 chains. Right, positions of unreduced proα(V) chain dimers (β), type IV procollagen trimers, and proα(V) chains. Note that the proα2 chain and a portion of the proα1(V) chains participate in complexes stabilized by interchain disulfide bonds. The slight differences in mobility of the monomeric proα1, proα(V), and type VIII-like chains in the presence and absence of DTT are attributed to intrachain disulfide bonds which limit unfolding of the chains in SDS buffer. Lower, subunit compositions of the various disulfide-bonded components in Lane 2 were analyzed by 2-dimensional SDS-PAGE. Unreduced proteins were resolved by SDS-PAGE in the first dimension (Long Arrow 1). Proteins in the gel slices were reduced with DTT and resolved by SDS-PAGE in the second dimension (Long Arrow 2). Left, internal standard of reduced medium proteins loaded in a well. Components migrating near the top of the gel prior to reduction give rise to chains with M, approximately 180,000, consistent with type IV procollagen (see below). The reduced dimeric β-components give rise to subunits which comigrate with reduced proα1(V) and proα2(V) chains. The proα(V) component appears heterogeneous and gives rise to proα1 chains, as well as a minor component which comigrates with proα2(V). Lower right, polydisperse type VIII-like band lacking interchain disulfide bonds and migrating near the “diagonal” (arrow). Note: left, components migrating out of the first dimension gel have slightly lower apparent mobility than the standards which were loaded in a well.
analysis of the chromatographically purified procollagen were similar to those reported for radiolabeled type V collagen synthesized by rhabdomyosarcoma cells (i.e., approximately 0.4) (16). (e) The type V collagens bound tightly to DEAE-cellulose at pH 8.2 and eluted at a salt concentration comparable to that previously reported for human type V PC (16, 21) (Fig. 5, left). When the bound components were rechromatographed on CM-cellulose under denaturing conditions, two major peaks were observed (not shown). The first peak contained predominantly proα1 and proα2(V) chains; whereas the second peak, which accounted for approximately two-thirds of the total radioactivity, contained disulfide-bonded components which gave rise to proα1(V) and proα2(V). Right, positions of α1(V) and α2(V).

Fig. 4. Cyanogen bromide peptide maps of radiolabeled proteins accumulating in the culture medium of C13. [3H]Proline-labeled medium proteins were concentrated by precipitation with ammonium sulfate, dialyzed versus 0.1 M acetic acid at 4°C in the presence of pepstatin, and lyophilized. A parallel aliquot was digested with pepstatin for 24 h at 4°C; the digest was lyophilized following inactivation of pepstatin with pepstatin. Proteins were resolved by SDS-PAGE on a 0.75-mm slab gel with or without prior reduction with DTT (Long Arrow 1). Individual lanes were cut from the gel and incubated with CNBr in 70% formic acid. After digestion the gel slices were horizontally positioned above a 1.5-mm 5%/10% slab gel and the resultant peptides were resolved by SDS-PAGE in the second dimension (Long Arrow 2). Left, pepstatin digest, unreduced in both dimensions; components comigrated with authentic human type V collagen chains. Center, medium proteins, unreduced in both dimensions. Right, medium proteins, reduced in both dimensions. Short arrows labeled 1, 2, 3, and 4 identify proα1(V), proα1(V), proα2(V), and the type VIII-like chains, respectively. Right, α1(I) and α2(I) CNBr peptide standards. Note that the proα1(V) and proα2(V) (center and right) chains show extensive homologies with the α1(V) and α2(V) chains (left); also note the presence of disulfide-bonded dimeric components (β, center) that contain α1(V) or α2(V)-derived peptides.

Minor collagenase-sensitive components consistent with type IV PC were also identified. The reduced components migrated smaller than interstitial proα chains on SDS-PAGE, migrated as disulfide-bonded trimers prior to reduction, did not bind to DEAE-cellulose under conditions in which types I, III, and V procollagens are known to bind, and were selectively coprecipitated with polyclonal antibodies to human type IV collagen. Limited pepsin digestion yielded high M, disulfide-bonded fragments which migrated near the position of intact proα1(I) chains (e.g., see Fig. 6, lane 3); the pepstatin-resistant components eluted from CM-cellulose near the position of α1(I). Finally, the major CNBr peptides showed homology with authentic type IV procollagen peptides (not shown).

Indirect immunoprecipitation assays revealed no evidence of
Fig. 5. Chromatographic purification of C13 collagens. Left, confluent cultures of C13 cells (6 100-cm² plates) were labeled for 24 h with 20 µCi/ml L-[2,3-³H]proline. The medium was clarified by centrifugation; collagens were precipitated with ammonium sulfate and chromatographed on DE52 cellulose as described. Pooled fractions were dialyzed, lyophilized, and examined by SDS-PAGE and fluorography. Inset: Lane 1, unbound fractions, Peak 1, unreduced; Lane 2, unbound fractions, Peak 1, reduced; Lane 3, bound fractions, Peak 2, reduced. Peak 1 contained type VIII collagen and type IV procollagen; Peak 2 contained type V procollagen. Middle, unbound fractions from DE52 dialyzed and chromatographed on CM-52 cellulose under denaturing conditions as described. Peak 2 fractions were examined by SDS-PAGE on a 3%/6% slab gel as above (Inset, left Lane). An aliquot was dialyzed, lyophilized, and subjected to CNBr mapping as in Fig. 4; peptides were resolved on a 5%/12.5% slab gel (Inset, right Lane). Right, position of an internal a(I)-CB7 standard. The map was identical to that observed for type VIII in CNBr digests of unfractionated medium proteins (Fig. 4). Right, unbound fractions from a separate DE52 run were concentrated by ultrafiltration, dialyzed versus 2 M guanidine-HCl, 50 mM Tris-HCl, pH 7.5, denatured by heating for 20 min at 50°C, and chromatographed on 6% agarose. SDS-PAGE of peak fractions revealed a single component of M₃ 180,000 (type I collagen standards); fractions near the void volume (Vₐ) contained disulfide-bonded components consistent with type IV procollagen.

Fig. 6. Type VIII collagen. Cultures of C13 cells were labeled with [³H]proline as in Fig. 2. Radiolabeled culture medium proteins were concentrated and chromatographed on DEAE-cellulose as in Fig. 5A. Peak 1 fractions (unbound) were pooled, dialyzed, and lyophilized; aliquots were incubated with bacterial collagenase or pepsin as described. Proteins were reduced with DTT, resolved by SDS-PAGE on a 3%/6%/10% slab gel, and visualized by fluorography. Lane 1, Peak 1 fractions; Lane 2, collagenase digest (C) of Peak 1; Lane 3, pepsin digest (P) of Peak 1. Note sensitivity of the type VIII-like collagen to pepsin; also note the presence of minor pepsin-resistant components derived from type IV procollagen (Lane 3).

Characterization of Clone 22. Clone 22 was characterized by a relatively uniform population of cells which exhibited a bipolar or fusiform morphology by phase-contrast microscopy (Fig. 1, right). The cells had abundant cytoplasmic intermediate filaments, a paucity of rough endoplasmic reticulum, and no evidence of junctional complexes by transmission electron microscopy. The clone had a stable aneuploid DNA index of 1.4 and a comparatively slow population doubling time of approximately 61 h. However, the clone appears to be “immortal” and shows no morphologic evidence of “senescence” or decreased growth rate after 2 yr and >20 transfers. Like C13, C22 did not grow in soft agar or form tumors in nude mice and elaborated a microfibrillar extracellular matrix.

Despite the somewhat “fibroblastic” morphology of C22 by light microscopy, it showed little evidence of collagen synthesis, consistent with the paucity of rough endoplasmic reticulum by electron microscopy. Collagenous proteins accounted for approximately 15% of the nondialyzable protein in the medium, and 2–3% of the nondialyzable radioactivity in the cell layer as assessed by radioactive hydroxyproline assay. SDS-PAGE revealed a minor collagenous component migrating near the position of proc(I) standards (Fig. 7, compare lanes 1 and 2). Following pepsin digestion, a single nondisulfide-bonded pepsin-resistant fragment was identified which comigrated with a(I) on SDS-PAGE consistent with type I trimer (or less likely, type II collagen) (Fig. 7, lane 3) (12, 28). There was no evidence of types I, III, IV, or V procollagen in the medium, and only faint pepsin-resistant components migrating near the position of a(I) chains were identified in the cell layer (not shown).

The major high M₃ collagen, radiolabeled component secreted by C22 cells was disulfide bonded and migrated on SDS-PAGE near the position of FN following sulfhydryl reduction (M₃ 230,000). The component was precipitable with monospecific antibodies to human plasma FN (Fig. 7, lanes 4 and 5), eluted from DEAE-cellulose near the position of fibronectin synthesized by human HT-1080 cells, and bound to inactivated Staphylococcus aureus (not shown). No laminin was identified by indirect immunoprecipitation. The biosynthetic phenotype of C13, C22, and several other clones is summarized in Table 1.

DISCUSSION

These studies demonstrate heterogeneity in the production of specific extracellular matrix macromolecules by several phenotypically distinct subpopulations of lung tumor cells in vitro.
INTRATUMOR HETEROGENEITY

In particular, JH-17-C13 was shown to secrete at least three genetically distinct collagens (V, IV, and a type VIII-like collagen), whereas JH-17-C22 synthesized fibronectin and a minor collagenous component presumptively identified as type I trimer.

Stone et al. have presented data that indicate that the parent cell line, JH-17, was phenotypically and genetically heterogeneous at the time of initial isolation and have shown that the various aneuploid subpopulations are stable with respect to DNA content. Although we were unable to obtain suitable tissues for biochemical characterization of tumor matrix at different anatomic sites, or for immunohistochemical localization of specific matrix antigens, the observed heterogeneity in production of matrix macromolecules by JH-17 clones in vitro provides strong evidence for intratumor heterogeneity in the production of extracellular matrix macromolecules.

The use of cell culture techniques and flow cytometric analysis have allowed us to exclude a contribution of nonneoplastic diploid cells to the heterogeneity of biosynthetic phenotypes. Although the precise derivation and histogenetic relationship of the various clones remain uncertain, the available data strongly suggest that the spindle cell clones, C22 and C26, are truly neoplastic and not simply reactive mesenchymal contaminants. Both clones possessed discrete and stable aneuploid DNA indices, and the biosynthetic phenotype was different from that usually observed for fibroblasts or myofibroblasts isolated from granulation tissue or desmoplastic stroma (i.e., with a predominance of types I, III, and V collagen) (29, 30). As previously indicated, C22 and C26 have shown no morphologic evidence of “senescence” or decrease in growth rate during prolonged culture in vitro. The absence of a desmoplastic reaction in histologic sections of tumor obtained at autopsy and the virtual absence of diploid cells in the initial isolates from pleural fluid also argue against derivation of these cells from a reactive or “transformed” stroma. Since cells with identical DNA contents and similar light microscopic morphologies were observed in four separate isolates of pleural fluid, it seems highly unlikely that the tumor cells arose by mutation during culture in vitro.

These observations strongly support our contention that the progenitors of C22 and C26 contributed to the cellular diversity of the tumor in vivo.

There was no obvious correlation between the production of specific matrix macromolecules and clonogenicity or tumorigenicity of the various clones. For example, clones C2 and C4 were clonogenic and formed tumors in nude mice but were virtually identical to C13 in their profile of radiolabeled, high $M_w$ secreted proteins. However, it will be important to compare the various clones with respect to their absolute levels of matrix protein production (e.g., by immunoassay). It will also be interesting to compare the expression of specific matrix receptors (e.g., fibronectin and laminin) for the tumorigenic and nontumorigenic clones.

Although the tumor was highly invasive and widely metastatic in vivo, we have not detected significant levels of active or latent type IV collagenase activity in culture medium (9, 31). We are presently looking for possible clonal differences in the levels of proteinase inhibitors that might influence type IV collagenolytic activity and for differences in the production or activity of other matrix-degrading enzymes such as interstitial collagenase.

The histogenesis of JH-17 remains uncertain, despite extensive characterization of the cells and their secreted matrix components. Insofar as many “large cell undifferentiated” tumors of the lung show convincing morphologic evidence of structural (i.e., squamous) and/or secretory differentiation, our

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Table 1 Phenotypes of representative JH-17 clones

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* DNA index relative to diploid DNA content as determined by flow cytometry following staining with propidium iodide. The JH-17 parent line contained two major populations with indices of 1.4 and 1.6. Indices of clones have remained essentially constant during 20–30 passages in vitro.

† Morphology: E, epithelial; F, fibroblastic.

‡ Tumors: i.p./s.c. injection.

§ Collagen types in culture medium as identified by SDS-PAGE and peptide mapping.
results further emphasize the heterogeneity of this diverse group of primary lung tumors. Although type V and/or IV collagens are deposited in the basement membrane zone of most epithelia, including lung, both components may be associated with the basal lamina or pericellular matrix of normal, as well as neoplastic, mesenchymal cells (e.g., endothelium, skeletal muscle, smooth muscle). Type V collagen is also widely distributed in interstitial connective tissues. Type VIII collagen has been reported to be synthesized by cultured endothelial cells, astrocytomas, a variety of carcinomas, including a purported alveolar cell carcinoma of the lung, but has not been identified in cultures of most normal epithelial strains or mesenchymal tumor cell lines (25). Alitalo et al. (32) have suggested that the profile of secreted matrix macromolecules may be used to detune the histogenesis of various tumors; however, our observations and results of others suggest that such an approach may be unreliable or misleading.

There is evidence that the growth, morphology, and biosynthetic phenotype of certain neoplastic cells may be influenced by interactions with extracellular matrix or purified matrix macromolecules in vitro (e.g., 33–35). This suggests that interactions of tumor cells with tumor- or stromal-derived extracellular matrix may influence the behavior of tumor cells in vivo. The observed heterogeneity of matrix macromolecules synthesized by distinct subpopulations of neoplastic cells raises the possibility of dynamic and reciprocal interactions between tumor cells and matrix which may be further altered as a consequence of tumor progression, growth, cell selection, invasion, and metastasis. Thus, temporal or regional variations in the biosynthetic phenotype of tumor cells may alter the character of the intratumor matrix, thereby modifying the behavior of host and tumor cells, further contributing to the evolution of the tumor.

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