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Growth of lung cancer cells in three-dimensional microenvironments reveals key features of tumor malignancy†

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Abstract

Cultured human lung cancer cell lines have been used extensively to dissect signaling pathways underlying cancer malignancy, including proliferation and resistance to chemotherapeutic agents. However, the ability of malignant cells to grow and metastasize *in vivo* is dependent upon specific cell-cell and cell-extracellular matrix (ECM) interactions, many of which are absent when cells are cultured on conventional tissue culture plastic. Previous studies have found that breast cancer cell lines show differential growth morphologies in three-dimensional (3D) gels of laminin-rich (lr) ECM, and that gene expression patterns associated with organized cell structure in 3D lrECM were associated with breast cancer patient prognosis. We show here that established lung cancer cell lines also can be classified by growth in lrECM into different morphological categories and that transcriptional alterations distinguishing growth on conventional tissue culture plastic from growth in 3D lrECM are reflective of tissue-specific differentiation. We further show that gene expression differences that distinguish lung cell lines that grow as smooth vs. branched structures in 3D lrECM can be used to stratify adenocarcinoma patients into prognostic groups with significantly different outcome, defining phenotypic response to 3D lrECM as a potential surrogate of lung cancer malignancy.

Insight, innovation, integration

The high incidence and poor prognosis of lung cancer has prompted substantial research towards identification of key effectors of lung cancer progression and metastasis which could be targeted therapeutically. A significant component of this research has involved the use of lung cancer cell lines, as these have been found to reflect many key genetic characteristics of the tumor of origin even after extensive culture. We demonstrate here that assay conditions previously shown to promote development of tissue-specific phenotypic characteristics in other cell types can be used with lung cancer cell lines to identify transcriptional alterations which are associated with response to 3D lrECM and which are predictive of lung adenocarcinoma patient prognosis. Our study describes an experimentally tractable model system in which potential effectors of lung cancer progression can be readily evaluated.

Introduction

Lung cancer is the leading cause of cancer death in the United States, and recent advances in early detection and improvements in treatment have had only a limited impact on overall

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prognosis.¹ Non-small cell lung carcinoma (NSCLC) accounts for approximately 80% of lung cancer cases and has an overall 5-year survival rate of approximately 15%;² accordingly, there is significant need for new therapeutic approaches for treating NSCLC patients. Development of conditions for long-term propagation of isolated lung cancer cells in culture has led to more than 150 well characterized and widely distributed cell lines.³ As analyses of genomic alterations has shown that lung cancer cell lines often closely resemble the tumors from which they were derived⁴, these cell lines represent potentially powerful tools both for identification of key processes involved in disease progression and as models for evaluating potential therapeutic strategies. However, the key features of malignancy, the ability to invade beyond the boundaries of the tumor and to metastasize to distant sites, are dependent upon cell-cell and cell-ECM interactions that are not well modeled in the conventional tissue culture conditions used to generate and propagate lung cancer cells.⁵ Growth in 3D IrECM assays has been found to model many of these interactions and has been used to identify tumor-associated processes which can be targeted to reduce cancer cell malignancy.⁶ Development of optimized methods for culturing mammary epithelial cell lines in 3D IrECM has revealed that nonmalignant breast cells develop into organized, growth-arrested acinar structures, whereas malignant cells continue to proliferate into disorganized cell masses, demonstrating that breast epithelial cell response to 3D IrECM reveals intrinsic characteristics.⁷ While the response of lung cancer cell lines in similar assays has not been as well characterized, an earlier comparison of normal bronchial tissue and three aggressive lung cancer cell lines in 3D IrECM revealed that the normal cells formed smooth, spheroid agglomerates, while cancer cells formed branching structures and invaded normal lung tissue explants.⁸ These results suggest that differential response of nonmalignant and malignant lung cancer cells to 3D IrECM could be used to define malignancy-associated pathways.

Transcriptional profiling of tumor biopsies from lung cancer patients has been used to identify biomarkers or sets of biomarkers that can be used to predict clinical outcome,⁹ to identify molecular markers of invasion¹⁰ and to classify lung cancer into distinct subtypes.¹¹ Such studies can identify clinically useful prognostic features. However, even highly prognostic transcripts may not be directly associated with the processes leading to better or poorer outcome, and thus may not represent clinically useful therapeutic targets. By contrast, a model system which allows both identification of prognostic biomarkers as well as evaluation of the consequences of activating or suppressing specific pathways on the malignant cell characteristics might be a better approach to identify potential therapeutic targets. Such studies have been pursued for breast cancer: transcriptional profiling of a panel of breast cancer cell lines in 3D IrECM revealed characteristic gene alterations associated with colony morphology,¹² and a transcriptional signature associated with cells that developed into a rounded, organized, and growth-arrested colony morphology was found to predict clinical outcome for breast cancer patients.¹³ As these studies employed in a highly tractable model system in which transcript expression can be easily modulated, evaluation of the impact of targeting specific pathways on the malignant growth phenotype could be easily assessed and used as an indicator of therapeutic potential. To test whether a similar relationship exists between lung cancer growth morphology in 3D IrECM and clinical outcome, we performed transcriptional profiling of lung cancer cell lines grown on conventional tissue culture plastic and in 3D IrECM. We identified transcriptional patterns that distinguished growth on plastic from growth in 3D IrECM. We also identified a transcriptional signature that distinguished cells forming smooth, round colonies in 3D from those with a branching, invasive morphology, and determined that this signature could separate patients with lung adenocarcinoma into better, intermediate, and poorer outcome categories. These results demonstrate that growth of lung cancer cells in 3D IrECM can be used to investigate clinical characteristics of lung cancer progression and that these

experimental models can be employed for discovery of novel prognostic biomarkers as well as evaluation of pathways potentially amenable to targeted therapies.

Results

3D IrECM growth characteristics of lung cancer cell lines

Nine commonly studied lung cancer cell lines (Table 1) were grown either on tissue culture plastic (2D) or in 3D culture on a thin layer of Matrigel covered with 2 % Matrigel dissolved in media ('on top' method).⁷ Cells were plated at equal density (5.0×10^4 per well) grown for five days. This time point was selected to provide distinct growth morphologies (Figure 1) while maintaining uniform proliferative status (assessed by Ki-67 staining, Supplemental Figure 1), so that subsequent transcriptional analyses would not be complicated by differential cell proliferation. Based on visual observation of growth patterns of lung cancer cells cultured in 3D IrECM, we classified the cells into two broadly distinct growth morphologies: smooth and branched (Fig. 1A). Smooth cell lines included H1437, H2170 and A549 (and H358, H460, not shown), which formed round or oval cell aggregates with strong cell-cell adhesion, and did not invade into the Matrigel. In contrast, H23 and H1703 (and H661, not shown) cell lines exhibited branched morphology, characterized by elongated cell bodies, and invasive protrusions. Chago-K1 cells developed into a distinct, grape-like morphology of poorly adhesive but noninvasive colonies. Immunofluorescent staining for filamentous actin and E-cadherin immunofluorescence showed distinct junctional staining in the smooth cells, while cells with the branched morphology generally showed cytoplasmic actin and little or no E-cadherin (Fig. 1B).

Analysis of gene expression of lung cancer cells in 2- and 3-dimensions

Gene expression profiles of seven of the lung cancer cell lines grown on conventional tissue culture plastic (2D) and in 3D IrECM (3D) were analyzed using Affymetrix GeneChip Human Genome U133 Plus 2.0 arrays. Two independent 3D culture samples were collected for each cell line for this experiment; replicates were not averaged but were treated as individual samples. We identified 228 transcripts that were significantly differentially expressed ($p < 0.05$, ANOVA) between samples grown in 2D and 3D (Fig. 2A; Supplemental Table 1); of these, 208 were downregulated in 3D as compared to 2D, while 20 were upregulated in 3D. We used Gene Ontology annotations¹⁴ to determine whether specific molecular functions were overrepresented in the set of genes differentially expressed in 3D as compared to 2D (Supplemental Table 2). Among six biological process Gene Ontology classifications, genes associated with GO:50896 (response to stimulus) were apparently overrepresented (just missing statistical significance, $p = 0.0797$); of the 27 differentially expressed genes annotated to GO:50896 (Figure 2B), 21 genes were associated with GO:6950 (response to stress; $p = 0.00439$) and 14 genes with GO:9719 (response to endogenous stimulus; $p = 4.32 \times 10^{-6}$). The other gene ontology classifications did not reach statistical significance due to the small number of identified genes. That the majority of the differentially expressed genes were downregulated in 3D in combination with the significant association with response to stress and stimulus ontology categories suggests that culture on tissue culture plastic substrata leads to activation of common transcriptional programs in lung cancer cell lines with widely varying morphologies in 3D IrECM (Figure 1) and different tumor characteristics (Table 1).

We subjected the list of differentially expressed genes to a NextBio (www.nextbio.com) meta-analysis, and identified data sets that were significantly associated with our list. Significant overlap was observed with data sets from differentiated vs. undifferentiated Caco-2 epithelial colorectal cancer cells ($P = 3.4 \times 10^{-49}$, Supplemental Fig. 2A)¹⁵ and from polarizing differentiating MCF10A cells in a Transwell model ($P = 4.5 \times 10^{-45}$; Supplemental

Fig. 2B),¹⁶ consistent with the notion that even cells from long-term established lung cancer cell lines adopt a more differentiated phenotype when grown in 3D IrECM.

Analysis of gene expression of smooth vs. branched lung cancer cell lines in 3-dimensions

Culturing lung cancer epithelial cells in 3-dimensional conditions resulted in formation of distinct morphologies that grouped the majority of evaluated cell lines into smooth and branched morphologies. Gene expression profiles of seven lung cancer cell lines grown in duplicate experiments in 3D were analyzed. Comparison of transcriptional patterns between 4 cell lines categorized as smooth and 3 cell lines categorized as branched resulted in identification of 200 transcripts that were significantly differentially expressed ($p < 1e-5$, ANOVA); of these, 63 transcripts were upregulated in branched morphology lines relative to smooth morphology cell lines, while 134 were downregulated in branched cell lines (Fig. 3A, Supplemental Table 3). We again used Gene Ontology annotations to classify differentially regulated genes (Supplemental Table 4), finding again that the most overrepresented biological process ontology category in our geneset was GO:50896 (response to stimulus; $p = 0.00199$). Of the 34 genes annotated to GO:50896 (Fig. 3B), the most overrepresented subgroup was GO:9607 (response to biotic stimulus), consistent with the differential response of the smooth and branching morphology cell lines to the IrECM tumor-derived microenvironment. NextBio meta-analysis of the transcripts differentially expressed between smooth and branching morphologies showed significant overlap with data sets from lung cancer cells cultured with tobacco smoke condensate vs. controls ($p = 1.2e-10$)¹⁷ (Supplemental Fig. 3A), as well as normal bronchial epithelial cells exposed cigarette smoke vs. non-exposed ($P = 3.5e-10$)¹⁸ (Supplemental Fig. 3B), suggesting that transcriptional programs induced by cigarette smoke, a known lung cancer carcinogen, might be associated with pathways controlling the invasive, branching morphology.

A 20-gene 3-dimensional signature is associated with lung cancer patient outcome

To assess the clinical relevance of the transcriptional patterns associated with the differential growth morphologies of the lung cancer cell lines, we defined a geneset containing the 20 most significantly differentially expressed (ANOVA, $P = 5e-10$) transcripts between the smooth and branching cell lines, and used this 20 transcript geneset to investigate a group of lung adenocarcinoma patients in the Duke cancer data set;¹⁹ this was facilitated by the fact that the Duke data set also had been analyzed using Affymetrix GeneChip Human Genome U133 Plus 2.0 arrays. Kaplan-Meier survival analyses performed for each of the individual transcripts in the dataset revealed that 7 transcripts were able to classify the patients into statistically significant better and poorer prognosis groups: MLPH-1 ($p = 0.0087$), RIN2 ($p = 0.036$), USP46 ($p = 0.037$), MGST1-1 ($p = 0.0036$), MGST1-2 ($p = 0.0011$), MGST1-3 ($p = 0.004$), LTBR ($p = 0.09$), and SLC27 ($p = 0.032$). (Supplemental Fig. 4). When we used expression levels of all 20 transcripts for hierarchical cluster analysis of the adenocarcinoma data set, we found that the patient data assembled into three groups, named here A, B and C, with 20, 24 and 14 patients per group, respectively (Fig. 4). While there was no statistical difference for average patient age or distribution of patient gender between the three subgroups (not shown), there was a substantial difference in the cancer stage, as stage 1 patients predominated group A, but progressively decreased in number in groups B and C, concomitant with an increasing number of more advanced cancers (Supplemental Fig. 5). Consistent with this distribution, analysis of the 3 groups by Kaplan-Meier analysis revealed a statistically significant difference ($p = 0.0053$) in outcome between the three groups (Fig. 5). Disease-free survival at one year was 100% for patients in group A, but dropped to 80% and 40% for patients in groups B and C, respectively. Strikingly, although group C contained mostly patients with late stage disease diagnoses, five of them were diagnosed with Stage 1, 1A, or 1B disease; of these five, three showed relapse at 2, 5, and 11 months.

These results indicate that assessment of prognosis using the 20 transcript geneset could potentially provide additional information beyond clinical-pathological parameters for patient diagnosis and assessment of treatment options.

Discussion

Currently, the preferred clinical method for assessing lung cancer prognosis and defining treatment decisions is the TNM staging system, which incorporates parameters of tumor size, spread to regional lymph nodes, and metastasis to distant sites.²⁰ Patients classified as stage 1 (those with invasive cancer but no spread to regional lymph nodes or distant metastasis) are generally treated by surgical resection only, although disease relapse rates for these patients can be as high as 30%, and it may be that some of these patients could benefit from chemotherapy. Stage 2 patients (with spread to regional lymph nodes but no distant metastasis) are often treated with adjuvant chemotherapy, although parameters for which stage 2 patients would best benefit from this additional treatment have not been defined. Transcriptional profiling studies of clinical datasets have been pursued towards identifying prognostic gene signatures that can be used to appropriately individualize treatment for early stage NSCLC patients.⁹ Each of these studies have produced a different panel of diagnostic transcripts, with little overlap between the different signatures; this partially reflects differences in experimental and statistical methods,²¹ as well as the different characteristics of the analyzed patient datasets.²² Here, we have evaluated the potential of a geneset composed of the 20 transcripts which best differentiate cell lines that develop into smooth vs branched morphologies when grown in 3D IrECM. When applied to a clinical dataset of lung adenocarcinoma patients, the 20-transcript geneset was able to define three distinct prognostic groups with significant differences in patient outcome. While three of the five patients (60%) categorized as stage 1 in group C showed relapse with an average time to relapse of six months, only 5/13 (38%) of the stage 1 patients in group A showed relapse at an average time of 36 months; thus, the 20-transcript geneset provides additional prognostic ability beyond conventional staging methods. While the limited sample size precludes analysis of statistical significance, it is remarkable that a geneset that was derived from a completely different type of data is nevertheless associated with patient outcome. When considered in combination with previous studies showing that genesets derived from breast cancer cell lines grown in 3D IrECM can also be prognostic for breast cancer disease outcome,¹³ these results further reinforce the notion that cancer cell lines retain critical clinical information about the tumors from which they were derived that is revealed in a microenvironment that models the cellular interactions found in tumors.^{6, 23} It may be that additional refinements in the culture model could reveal additional phenotypic information, as with a recently published system which incorporated tissue fibroblasts and air-liquid interfaces in which immortalized nonmalignant bronchial epithelial cells were shown to develop along different differentiation lineages.²⁴

Additional insight can also be found by examining individual components of the 20-transcript geneset used to classify the clinical dataset. SMARCA2 (also known as Brahma, or BRM), is a component of the SWI/SNF chromatin remodeling complex. In the clinical dataset, SMARCA2 showed increased expression in the better prognosis group A, but decreased expression in the poorer prognosis groups B and C. These results are consistent with studies showing that loss of SMARCA2 is correlated with poor prognosis in lung cancer patients²⁵ and is a component of a breast cancer predictive signature.²⁶ MGST1 (microsomal glutathione S-transferase 1) is downregulated in group A and upregulated in groups B and C; MGST1 was previously shown to be overexpressed in lung tumors developing in transgenic lung cancer models.²⁷ That differences in MGST1 expression are by themselves prognostic of patient outcome (Supplemental Figure 4) as well as associated with altered morphological growth responses suggests that the 3D IrECM assay could be

used to test therapeutic approaches targeting MGST1 as well as the pathways controlled by them, using morphological reversion as a potential endpoint. More generally, high throughput application⁷ of the lung cancer cell lines with the 3D IrECM assay could be used to screen compound libraries to identify novel agents capable of reversion of the malignant branched phenotype; tumor reversion is an emerging approach with considerable potential to augment existing anticancer therapies.²⁸

It should be noted that not all of the members of the 20 transcript geneset were clearly associated with patient outcome. While some individual transcripts could be used to divide patients into high and low expressing groups with significantly different outcomes (e.g., MLPH1, MGST1, RIN2); in other cases, transcripts that were significantly differentially expressed between cell lines growing in 3D IrECM as smooth vs branching showed very low expression in the adenocarcinoma dataset (e.g., SLC27A2), or were not by themselves associated with differential outcome (e.g., HIPK2). However, even transcripts that did not by themselves predict outcome might nevertheless contribute to the prognostic characteristics of the 20 transcript geneset. Evaluation of manipulation of individual genes on growth morphology may provide a more definitive method for determining whether the differential expression in 3D is directly or indirectly related to the growth phenotype.

While there were many transcriptional alterations that distinguished cell lines showing smooth and branched morphology, there was also substantial overlap in transcriptional changes for all cell lines between growth on tissue culture plastic and growth in 3D IrECM. Gene expression analysis of lung cancer cells grown either in 2D or 3D culture revealed 228 differentially expressed transcripts that corresponded to 140 annotated genes. NextBio meta-analysis revealed that expression data of 2D vs. 3D grown cells negatively correlates with data from differentiated vs. undifferentiated Caco-2 cells,¹⁵ and with polarizing differentiating vs undifferentiated MCF10A cells.¹⁶ This indicates that the 3D IrECM-cultured cells are more similar to the differentiated phenotype of both cell lines, consistent with previous studies showing activation of tissue-relevant phenotypic characteristics for mammary epithelial cells grown in tissue-like context.^{23a} Among individual transcripts in the 2D vs. 3D grown cells, SERPINB6 was the most consistently upregulated by growth in 3D IrECM (avg. 2D/3D expression=0.01, $p=3.2e-11$; Supplemental Table 1). SERPINB6 belongs to a family of serine proteinase inhibitors clade B, and while its function has not been fully defined, a transcriptional study identified SERPINB6 as substantially and significantly upregulated during endothelial cell tubulogenesis in 3D IrECM,²⁹ indicating the potential existence of overlapping pathways linking lung cancer cell lines and endothelial cells. Another transcript that was consistently upregulated in cells grown on 3D vs. 2D was RAB11A (avg. 2D/3D expression=0.44, $p=0.015$; Supplemental Table 1), a GTPase involved in apical recycling endosomes in polarized epithelial cells,³⁰ implicating the transcriptional activation of molecules involved in directional protein sorting. Transcripts downregulated in 3D vs. 2D included PLK1 (Polo-like Kinase 1, avg 2D/3D expression=2.03, $p=0.00032$), a mitotic kinase which has been implicated as a regulator of growth into 3D IrECM,³¹ and MALAT 1 (Metastasis-associated lung adenocarcinoma transcript 1, avg 2D/3D expression=1.75, $p=0.0097$), a noncoding RNA associated with early-stage NSCLC³² and which has been found to control alternative splicing³³ and lung cancer cell motility.³⁴ While some transcripts showed increased expression in cells grown in 3D IrECM, the majority of significantly differentially expressed transcripts were downregulated in cells grown in 3D IrECM (Figure 2). How these transcriptional alterations are controlled in the lung cancer cell lines is unclear; however, previous studies have found activation of tissue-specific transcripts associated with general gene repression as a consequence of histone deacetylation in mammary epithelial cells grown in 3D IrECM,³⁵ consistent with models indicating broad-scale chromatin alterations induced by growth in tissue structures.³⁶ To what extent the specific molecular processes activated by 3D IrECM

in mammary epithelial cells mirror those induced in lung cancer cell lines remains to be discovered.

Conclusions

Cultured lung cancer cell lines retain many of the genomic alterations found in the tumors from which they were derived and can be easily accessible for experimental replication; use of culture models simulating key aspects of the tumor microenvironment, such as the 3D IrECM model used here, facilitate development of multicellular structures reflective of the phenotypic alterations controlling cancer cell malignancy. Our findings further emphasize the importance of cell-cell and cell-ECM interactions as effectors of cellular function, and demonstrate how these principles can be applied to identify novel cancer-associated processes that are potential targets for therapeutic intervention.

Experimental section

Cell culture

A549 cells were cultured in Ham's F12K medium with 10% FBS, 2 mM L-glutamine and 1.5 g/L sodium bicarbonate; H23, H358, H460, H661, H1703, H2170, and ChagoK1 cells were grown in RPMI supplemented with 10% FBS, 1 mM sodium pyruvate, 2mM L-glutamine, 10mM HEPES (Invitrogen), 4.5 g/L glucose and 1.5 g/L sodium bicarbonate. All media was also supplemented with 50 µg/ml gentamycin (all culture reagents from Invitrogen). Before plating for 3D IrECM cultures, cells were propagated for 7 days in 2D culture on plastic, and then seeded in growth media on top of a solidified layer of Matrigel (BD Biosciences), and then overlaid with a solution containing 2% matrigel, as was previously described.⁷ The cells were cultured for 5 days before fixation for immunofluorescence or harvesting RNA for transcriptional analysis.

Immunofluorescence

The 3D cultured cells were prepared for staining by subsequent washes with 15 % and 30 % sucrose solution in PBS, after which the cells were diluted in serum free culture media, and smeared on glass slides to air dry. The cells were fixed with 4 % formaldehyde, permeabilized with 0.1 % Triton X100 solution in PBS, and blocked for 1 hour in an immunofluorescence buffer (0.2 % Triton X100, 0.1 % BSA, 0.05 % Tween 20 in PBS) plus 5 % goat serum. Primary incubation with antibodies targeting Ki-67 or E-cadherin diluted 1:100 was performed for 2 hours. Secondary incubation used antibodies conjugated with Alexa Fluor 488, diluted 1:200 in blocking buffer and incubated for 1 hour. The cells were also stained with Hoechst 33342 1:10000 for 10 minutes, and Alexa fluor 594 phalloidin 1:500 for 30 minutes. All steps were performed at room temperature. All antibodies and cell staining reagents were from Invitrogen. Images were acquired using an Olympus IX71 fluorescent microscope. The percentage of Ki-67 positive cells with Hoechst stained nuclei was determined as an average staining of four fields of about 30–40 cells for each cell line.

Transcriptional analysis

After the cells were grown in either 2D or 3D culture for 5 days, RNA was isolated and analyzed using previously described methods.³⁷ Briefly, RNA was labeled and hybridized to Affymetrix GeneChip Human Genome U133 Plus 2.0 arrays (Affymetrix) at the Mayo Clinic core facility. The GCRMA function of the GeneSpring software (Agilent) was used for processing, normalization and background correction. For identification of genesets that substantially and significantly distinguished 2D vs. 3D or smooth vs. 'invasive', transcripts were filtered for 2-fold average expression differences between categories and analyzed for ANOVA using parametric tests with variances not assumed equal (Welch t-test) and

Benjamini and Hochberg False Discovery Rate multiple testing correction. Gene Ontology was analyzed using GeneSpring. The NextBio platform (www.nextbio.com) was utilized for meta-analysis of differentially expressed genes in order to identify significantly associated data sets. Kaplan-Meier survival curves were performed using GraphPad Prism.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Morphologies of lung cancer cell lines grown in 3D

A. Lung cancer cells adopt a flattened morphology when grown on tissue culture plastic (2D, top row), but can form smooth, round masses (H1437, H358, H2170, and A549), grape-like structures (ChagoK1), or invasive, branching structures (H23 and H1703) when grown in 3D IrECM cultures (3D, bottom row). Scale bar 50 mm. B. Expression of actin cytoskeleton (Actin, top row), E-cadherin (second row), cell nuclei (Hoechst, third row), and all three stains merged (Actin red, E-cadherin green, Hoechst blue; bottom row) of lung cancer cells grown in 3D. Scale bar 20 μm.

Figure 2. Analysis of transcripts differentially expressed in lung cancer cell lines grown in 2D and 3D

A. Hierarchical clustering of 228 transcripts that are significantly different ($p < 0.05$, ANOVA) between lung cancer cell lines grown on tissue culture plastic (2D) or in 3D IrECM (3D). Red, elevated transcript expression; blue, decreased expression. B. Gene Ontology (GO) analysis of genes distinguishing 2D and 3D cells as subset of GO:8150: biological_process.

Figure 3. Analysis of transcripts differentially expressed in lung cancer cell lines that grow as smooth or invasive morphologies

A. Hierarchical clustering of 200 transcripts that are significantly different ($p < 1e-5$, ANOVA) between lung cancer cell lines that grow as smooth or invasive morphology in 3D culture. Red, elevated transcript expression; blue, decreased expression. B. Gene Ontology (GO) analysis of genes distinguishing smooth and invasive morphology as subset of GO: 8150: biological_process.

Figure 4. Transcripts that best discriminate smooth vs. invasive morphology used to cluster lung cancer
The 20 transcripts that most significantly different ($p < 5e-10$) between lung cancer cells that grow as smooth or invasive were used to cluster 59 lung adenocarcinoma patients based on gene expression in Duke-Adeno dataset. Dendrograms on top and left represent degree of relatedness of individual samples (top) and gene probes (left). Rows, relative level of transcript expression (inset, color scale); columns, individual patients. Bottom plate contains age, gender, stage, survival (in months) and incidence information for individual patients. Top dendrogram branch colors represent different prognostic groups.

Figure 5. Transcripts that best discriminate smooth vs. invasive morphology classify lung cancer patients into differential outcome categories

The 20 transcripts that most significantly different ($p < 5e-10$) between lung cancer cells that grow as smooth or invasive morphologies cluster 59 lung adenocarcinoma patients into three groups with significantly different outcomes ($p = 0.0053$). Group A, 20 patients; group B, 24 patients; group C, 14 patients.

Characteristics of lung cancer cell lines used in study.

Table 1

Cell Line	3D morphology	Tumor Type	Mutational status		Cigarette usage	E-cad
			TP53	KRAS		
H1437	Smooth	AC	Mt	Wt	S	+
H358	Smooth	NSCLC	Mt	Mt	NS	+
H2170	Smooth	SCC	Mt	Wt	NS	+
A549	Smooth	AC	Wt	Mt	-	+
ChagoK1	Grape-like	BC	Mt	Wt	-	+
H23	Branching	AC	Mt	Mt	S	-
H1703	Branching	NSCLC	Mt	Wt	S	-
H460	Smooth	AC,LCC	Mt	Wt	NS	-
H661	Branching	LCC	Mt	Wt	NS	-

AC, Adenocarcinoma; NSCLC, Non Small Cell Lung Cancer; SCC, Squamous cell carcinoma; BC, bronchial carcinoma; LCC, Large cell carcinoma; Mt, mutant; Wt, wild type; S, smoker; NS, nonsmoker.
<http://www.sanger.ac.uk>; <http://www.atcc.org>