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Differential Expression of Critical Cellular Genes in Human Lung Adenocarcinomas and Squamous Cell Carcinomas in Comparison to Normal Lung Tissues[1]

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Abstract

The Atlas human cDNA expression array was used to evaluate gene expression profile changes in the genesis of human lung adenocarcinomas and squamous cell carcinomas. Gene expression changes between adenocarcinomas and squamous cell carcinomas were also analyzed. Of the 588 gene targets, 262 genes were expressed in these tissues and, of these, 45 genes were differentially expressed by at least two-fold in tumor tissues compared to corresponding normal tissues. Semiquantitative reverse-transcriptase polymerase chain reaction was used to confirm gene expression changes. Only those genes that reflected changes in >50% of the analyzed tissues were included in the final analysis. Ultimately, 26 genes were evaluated with 14 genes overexpressed and 12 genes underexpressed compared to matching normal lung tissues. Although similar expression changes were detected in adenocarcinomas and squamous cell carcinomas for most of the genes analyzed, some subtype-specific differences were also found. Genes encoding cell cycle regulators, intracellular signal transducers, cell receptor and adhesion molecules, growth factors, oncogenes, and apoptotic effectors were differentially expressed in this study. These gene expression changes may directly contribute to the initiation or progression of human lung cancer or may be secondary effects of the tumorigenesis process. Regardless, many of these differences may be useful in the diagnosis and/or treatment of this deadly disease.

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Keywords: non–small cell lung cancer, cDNA microarray, expression profile, differential changes, cancer genes.

Introduction

An estimated 553,400 Americans will lose their lives to cancer in the year 2001. Lung cancer is the most common type of cancer death among men and women in the United States, with an estimated 169,500 new cases and 157,400 deaths predicted for the year 2001 [1]. The molecular pathogenesis of human cancer involves the accumulation of genetic and epigenetic alterations in cancer-related genes [2,3]. Progression to lung cancer is believed to involve 10 to 20 alterations, including the activation of proto-oncogenes and the inactivation of tumor suppressor genes [4]. In addition to overriding growth control mechanisms, transformed cells must also avoid programmed cell death that can ensue when cell cycle checkpoint control is lost. Although most tumors have high rates of apoptotic death, cancer cells frequently inactivate components of apoptotic pathways and induce cell survival genes [5]. Additional alterations may include disruption of DNA repair genes and chromosomal fragile sites as well as activation of invasive, metastatic, and/or angiogenic factors. Genetic susceptibility may also contribute to the development of human lung cancer [4,6].

Lung cancers are broadly categorized into two histologic groups. Small cell lung cancer (SCLC) is diagnosed in approximately 20% to 25% of all lung cancer cases and non–small cell lung cancer (NSCLC) is diagnosed in the remaining 75% to 80% of cases [7,8]. NSCLC is further subdivided into three groups: 1) squamous cell carcinoma is characterized by rapidly growing epidermoid cells that produce keratin (~25%); 2) adenocarcinoma arises peripherally and is composed of cuboidal or columnar cells that can form glandular patterns and produce mucin (~30%); 3) large cell carcinoma consists of a heterogeneous mix of poorly differentiated cells that do not resemble cells from the other two categories (~10%) [7,8].

Cytogenetic and molecular studies have revealed different genetic alterations in these two histologic subgroups. For

Abbreviations: AR, amphiregulin; eph, erythropoietin-producing hepatocellular; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HGF, hepatocyte growth factor; ICAM1, intercellular adhesion molecule 1; IL2Rα, interleukin 2 receptor alpha; NSCLC, non-small cell lung cancer; RT-PCR, reverse-transcriptase polymerase chain reaction; SCLC, small cell lung cancer

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example, the expression frequency of *Bcl-2* is much higher in SCLC (75% to 95%) than in NSCLC (10% to 35%) [6,9-11]. Additionally, within NSCLC, squamous cell carcinomas express higher levels of *Bcl-2* more frequently than adenocarcinomas (25% to 35% vs. ~10%, respectively) [6,10,12,13]. Abnormal Rb protein expression is detected in over 90% of SCLC but only 15% to 30% of NSCLC [14-16]. $p16^{INK4a}$ inactivation is more frequently detected in NSCLC (~10% to 40%) and very rarely in SCLC (<1%) [6,17,18]. Activating point mutations in the ras gene family occur in 20% to 30% of adenocarcinomas and 15% to 20% of all NSCLC but in <1% of SCLC [6,19]. In contrast, the frequency of abnormal p53 expression detected by immunohistochemistry is ~40% to 70% for both SCLC and NSCLC [20-22]. Most studies have reported a higher frequency of abnormal expression of the p53 gene in squamous cell carcinomas compared to adenocarcinomas [6]. In SCLC, losses from chromosomes 1p, 3p, 5q, 17p, and 10q predominate [23,24]. Common alterations in NSCLC include 3p, 6q, 8p, 9p, 9q, 13q, 17p, 18q, 19p, 21q, 22q, as well as +7, i(5)(p10) and i(8)(q10) [23,25]. Genetic alterations appear to differ between SCLC and NSCLC and possibly even between NSCLC subtypes.

Using a human cDNA expression array, we determined gene expression profile changes between human lung NSCLCs and normal human lung tissues. We also analyzed the gene expression profile changes between human lung squamous cell carcinomas and adenocarcinomas with respect to their matching normal lung tissues. The cDNA expression arrays allow for rapid, high-throughput analyses of gene expression patterns in tissues. Analysis of differentially expressed genes in human lung tumors may reveal additional, unsuspected genes involved in lung tumorigenesis, provide novel diagnostic markers or chemopreventive targets, and/or implicate the involvement of other molecular pathways in cancer development.

Materials and Methods

Tissue Specimens

Frozen lung cancer specimens and matching normal tissues were obtained from Cooperative Human Tissue Network through The Ohio State University Department of Pathology. Fourteen pairs of clinical samples (seven squamous cell carcinomas with their normal controls and seven adenocarcinomas with their normal controls) were used in this study (Table 1). Frozen tumor tissues were microdissected to determine the tumor versus normal cell ratio for each specimen. Tissues were embedded in Tissue Tek OCT compound (VWR Scientific Products, West Chester, PA), cryostat sectioned, and stained with hemotoxin and eosin for microscopy. Tumor tissue sections corresponding to the microscopic sections containing $\geq$70% tumor cells were isolated and stored at -80°C for subsequent RNA isolation. Matching normal tissues were also microdissected to ensure that specimens consisted of purely normal lung tissue. Figure 1 shows the typical

Table 1. Description of Adenocarcinomas and Squamous Cell Carcinomas Used in This Study.

Patient No.	Description	Location	Diagnosis	Other Relevant Facts
8322	48Y, W, F	RLL	Invasive, moderately differentiated SCC	Smoker: 1 ppd×25 years; prior SCC of the lung treated with radiation; mother died of breast cancer
8326	72Y, W, F	RLL	Moderately differentiated SSC; vascular invasion; lymph node metastasis	Smoker: 1/2 ppd×30 years; prior SCC of the lung treated with chemotherapy×5
8599	62Y, F	RA Lung	Moderately to poorly differentiated SCC; pleural and intrapulmonary lymphatic invasion	Prior right lung cancer
8611	67Y, M	LUL	Moderately differentiated bronchogenic SSC with desmoplastic reaction	Atelectasis and inflammation with cavitation and hemorrhage
8706	74Y, M	LUL	Poorly differentiated SSC with extensive necrosis	Emphysematous change with focal chronic interstitial pneumonitis and fibrosis; atelectasis
94-10-A039	68Y, W, M	RUL	Moderately differentiated SCC with no evidence of keratin formation; adenosquamous in some areas	Moderate emphysema in normal lung tissue
94-11-A120	58Y, W, M	LLL	Poorly differentiated endobronchial SSC with lymph node metastasis	Prior asbestos exposure
5796	69Y, F	RUL	Moderately to poorly differentiated alveolar AdC with extensive central necrosis	Moderate lymphocytic host response
8252	70Y, W, F	RML	Poorly differentiated AdC with bronchioalveolar pattern at its periphery; lymph node metastasis	Prior cervical carcinoma but compatible with primary lung carcinoma
8606	52Y, W, F	LLL	Poorly differentiated AdC with lymph node metastasis	Smoker: 1.5 ppd×32 years; prior RU lobe tumor; post-obstructive chronic pneumonia
8607	45Y, W, F	LUL; LLL	Adenocarcinoma; mass present at hilum and junction of LU lobe and LL lobe	Brain metastasis
8641	68Y, B, F	RML	Moderately differentiated AdC invading into bronchus and vasculature	Tumor destroys and fills one bronchus; chronic bronchitis and centrilobular emphysema
8712	76Y, W, F	RUL	Moderately differentiated AdC with lymph node metastasis	History of skin cancer and primary RU lobe lung cancer
94-11-C066	51Y, M	RLL	Poorly differentiated AdC	Smoker: 1.5 ppd×30 years

Abbreviations: *AdC*, adenocarcinoma. *SCC*, squamous cell carcinoma. *W*, white. *B*, black. *F*, female. *M*, male. *Y*, years old. *ppd*, packs per day.
Location: first position: R, right; L, left; second position: U, upper; M, middle; L, lower; third position: L, lobe.

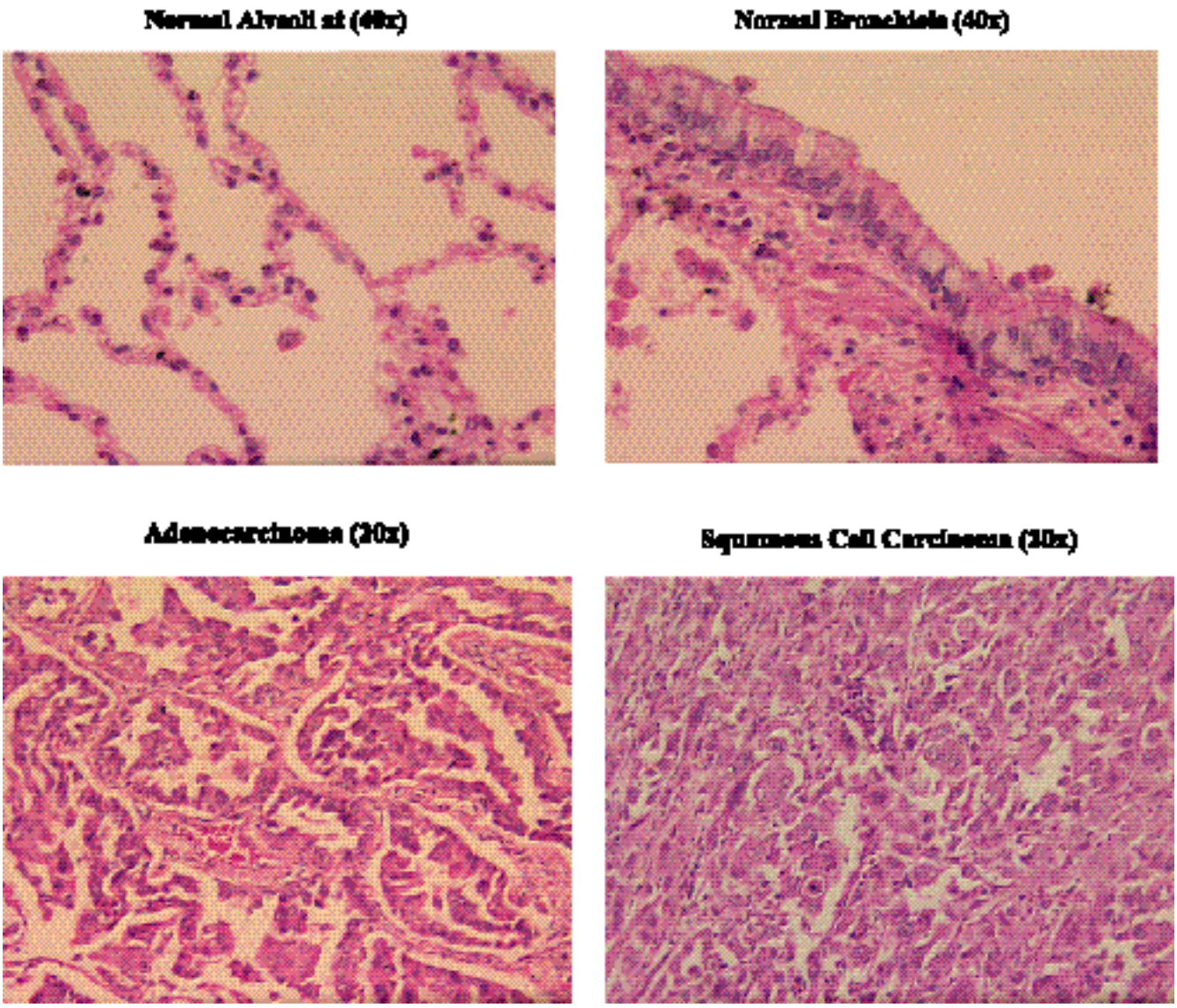

Figure 1. *Histology of normal and tumor tissues used in this study.*

morphology of normal alveoli and an adenocarcinoma as well as a normal bronchiole and a squamous cell carcinoma used in this study.

PolyA$^+$ RNA Isolation

Total RNA was extracted using TRI-Reagent according to standard protocol (Molecular Research Center, Cincinnati, OH). Briefly, after homogenization in 5.0 ml TRI-Reagent, 1.0 ml chloroform was added per sample, shaken vigorously, and centrifuged for 15 minutes at 4°C, 12,000*g*. The clear, upper phase containing total RNA was collected for isolation of the polyA$^+$ RNA fraction. One volume of aqueous phase was mixed with 0.1 volume of 1 M Tris (free base) and 0.8 volume isopropanol and stored at room temperature for approximately 5 minutes. Meanwhile, oligo(dT)-cellulose columns (Molecular Research Center) were washed with 1.0 ml binding buffer (0.5 M LiCl, 50 mM sodium citrate, 0.1% SDS). The RNA suspension was applied to the cellulose column to which the polyA$^+$ RNA adheres. The columns were then washed with 0.5 ml 75% ethanol and 1.0 ml binding buffer. Finally, the polyA$^+$ RNA fraction was eluted from the column with 0.6 ml elution buffer (1 mM sodium citrate, 0.1% SDS), quantitated by spectrophotometry, and precipitated with a final concentration of 0.3 M NaCl and 1.5 volumes isopropanol. Pellets were dissolved in nuclease-free water to a final concentration of 1 μg/μL.

cDNA Expression Array Hybridization

A total of 5 μg of polyA$^+$ RNA was reverse transcribed using 0.2$\times$CDS primer mix (Clontech Laboratories, Palo Alto, CA); 10 mM DTT; 1$\times$ reverse transcription buffer (50 mM Tris–HCl, pH 8.3; 75 mM KCl; 3 mM $MgCl_2$); 1 mM each of dATP, dTTP, and dGTP; 80 U RNAsin (Promega, Madison, WI); 50 μCi [α-^{32}P]dCTP and 800 units MMLV reverse transcriptase (Gibco BRL Life Technologies, Carlsbad, CA). The mixture was incubated at 37°C for 1 hour and then purified by centrifugation through G50 Sephadex columns, denatured at 95°C for 10 minutes, and hybridized to Atlas cDNA

expression arrays (Clontech Laboratories, Palo Alto, CA) for 16 to 20 hours at 42°C. Membranes were then washed twice, each for 10 minutes in 2×SSC, 0.1% SDS at room temperature, and twice, each for 30 minutes in 0.1×SSC, 0.1% SDS at 55°C, followed by exposure to Molecular Dynamics Phosphorimager screens (Sunnyvale, CA) overnight. Densitometry was performed using ImageQuant software.

Reverse Transcription PCR

Primers for each differentially expressed gene were designed and ordered from Gibco BRL Life Technologies. Each primer was diluted to 1 OD/100 μL and forward primers were then end-labeled with [γ-^{32}P]dATP using T4 polynucleotide kinase (New England Biolabs, Beverly, MA). A total of 4 μg normal/tumor tissue total RNA and 2.5 μg oligo-dT primer were denatured at 65°C for 10 minutes and placed on ice. To this mixture the following components were added: 1×reverse transcription buffer (50 mM Tris–HCl, pH 8.3; 75 mM KCl; 3 mM $MgCl_2$); 10 mM DTT; 1.0 mM of each dATP, dTTP, and dGTP; 100 μCi [α-^{32}P]dCTP or 1.0 mM dCTP; 60 U RNAsin (Promega); and 800 U MMLV reverse transcriptase (Gibco BRL Life Technologies). Reaction mixtures were incubated at 37°C for 1 hour. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control in each PCR amplification. The coamplification of the control cDNA and the target gene cDNA in tumor and normal tissues provided a means to control for PCR amplification and enabled the relative level of the target gene expression to be quantified. PCR reactions using 1 μL cDNA, 1×reaction buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 0.75 units of Taq DNA Polymerase (Promega), labeled forward primers, and unlabeled reverse primers were subjected to 18 to 24 cycles of amplification at 95°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute per cycle. To determine the linear range of each set of PCR reactions, a series of 3 to 4 PCR reactions using 18, 20, 22, or 24 cycles were performed for each target gene, and one of the reactions that fitted into the linear range was used for further quantitation. PCR products were then electrophoresed on

Table 2. Differentially Expressed Genes in Adenocarcinomas and Squamous Cell Carcinomas.

No.	Gene	GenBank No.	RT	RT Frequency
Oncogenes, tumor supressor genes, and cell cycle regulators				
1	protein tyrosine kinase c-kit	X06182	–	5/7S, 6/7A, 1S&1A(+)
2	c-jun	J04111	–	2/7S, 5/7A
3	protein tyrosine kinase receptor tyro3	D17517	–	5/7S, 5/7A
4	c-src	K03214	–	5/7S, 3/7A, 4S(+)
5	c-fgr proto-oncogene	M19722	–	5/7S, 4/7A, 1A(+)
6	TR3 orphan receptor	L13740	–	6/7S, 6/7A, 1S(+)
7	cdc25A	M81933	+	5/7S, 3/7A, 2A(–)
Stress response and modulators, effectors, and intracellular transducers				
8	ephrin type-A receptor 1	M18391	+	3/7S, 6/7A, 1S&2A(–)
9	LIM domain kinase 1 (LIMK-1)	D26309	+	3/7S, 4/7A, 1S(–)
10	ras-related protein RAB6	M28212	+	3/7S, 3/7A, 1S(–)
11	ras-related protein RAB2	M28213	+	0S, 5/7A
12	janus kinase 3 (JAK3)	U09607	+	4/7S, 3/7A, 1S&2A(–)
13	c-jun N-terminal kinase1 (JNK1)	L26318	+	4/7S, 3/7A, 1S(–)
14	ephrin A3 (EFNA3)	U14187	+	7/7S, 6/7A
15	transmembrane receptor PTK7	U40271	+	3/7S, 5/7A, 1S(–)
Apoptosis and DNA synthesis, repair, and recombination				
16	adenosine A1 receptor (ADORA1)	S56143	–	5/7S, 3/7A, 2S(+)
17	caspase 8	U60520	–	3/7S, 4/7A, 3A(+)
Receptors, cell surface antigens, and cell adhesion				
18	insulin receptor (INSR)	M10051	+	3/7S, 4/7A, 2S(–)
19	IL2Rα	X01057	+	4/7S, 7/7A
20	CD27L antigen receptor	M63928	+	5/7S, 5/7A
21	ICAM1	J03132	–	7/7S, 2/7A, 1S&1A(+)
Growth factors, cytokines, chemokines, and interferons				
22	HGF activator	D14012	+	4/7S, 6/7A, 2S&1A(–)
23	AR	M30704	–	6/7S, 5/7A, 2A(+)
24	vascular endothelial growth factor C	U43142	–	4/7S, 2/7A, 1S&1A(+)
25	platelet-derived growth factor A (PDGFA)	X06374	+	4/7S, 4/7A, 2S&1A(–)
26	macrophage inflammatory protein 2 alpha	X53799	–	5/7S, 6/7A

A, adenocarcinoma. *S*, squamous cell carcinoma. –, underexpressed; +, overexpressed compared to normal lung tissue from the same patient. *RT*, reverse transcription–PCR analysis.
RT Frequency, ratio of the number of tumors with differential expression over the total number of normal/tumor tissue pairs analyzed; S/A(+/–), differential expression detected in tumors that did not reflect what was found in the majority of normal/tumor tissue pairs.

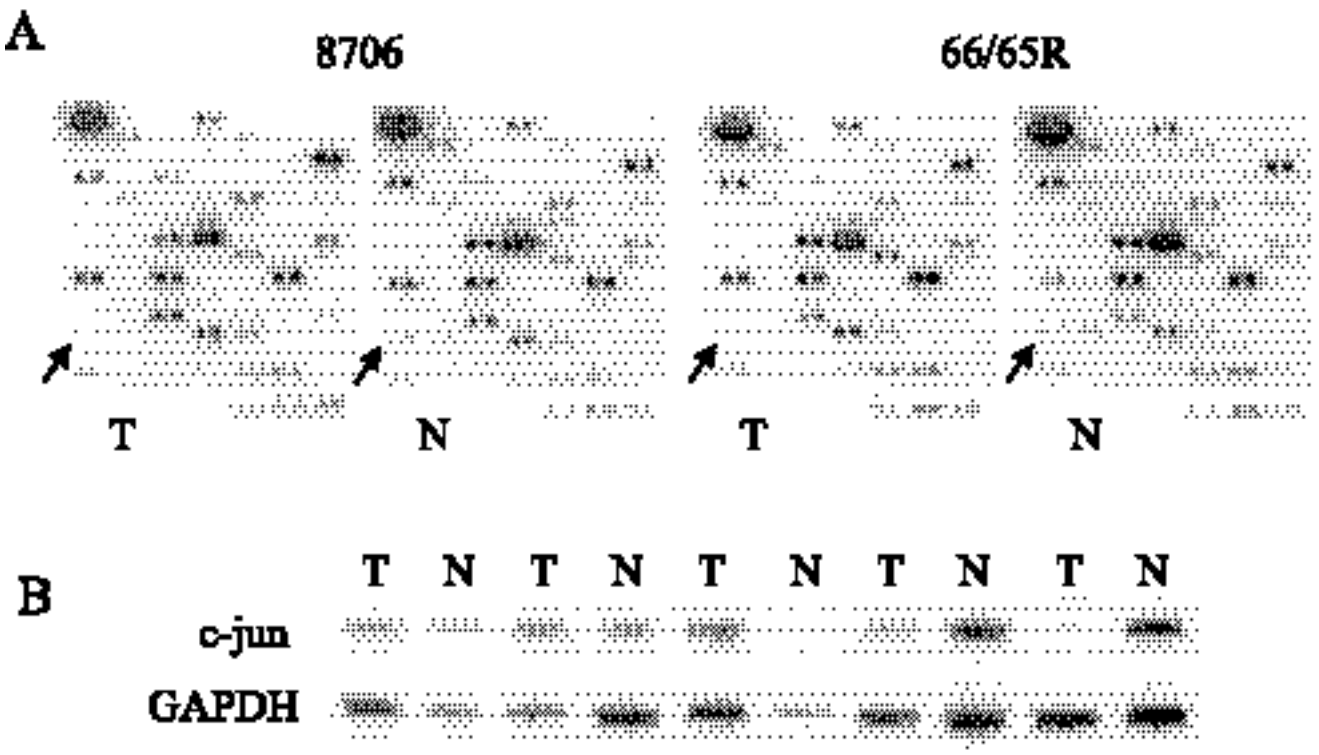

Figure 2. *Differential expression of c-jun. (A) cDNA expression array hybridization with the squamous cell carcinoma 8706 and its matching normal tissue and the adenocarcinoma 94-11-C066R (66R) and its matching normal tissue 94-11-C065R (65R). (B) RT-PCR confirmation. The first two pairs of tumor/normal tissues from the left are squamous cell carcinomas of the lung. The remaining three pairs of tumor/normal tissues are lung adenocarcinomas.*

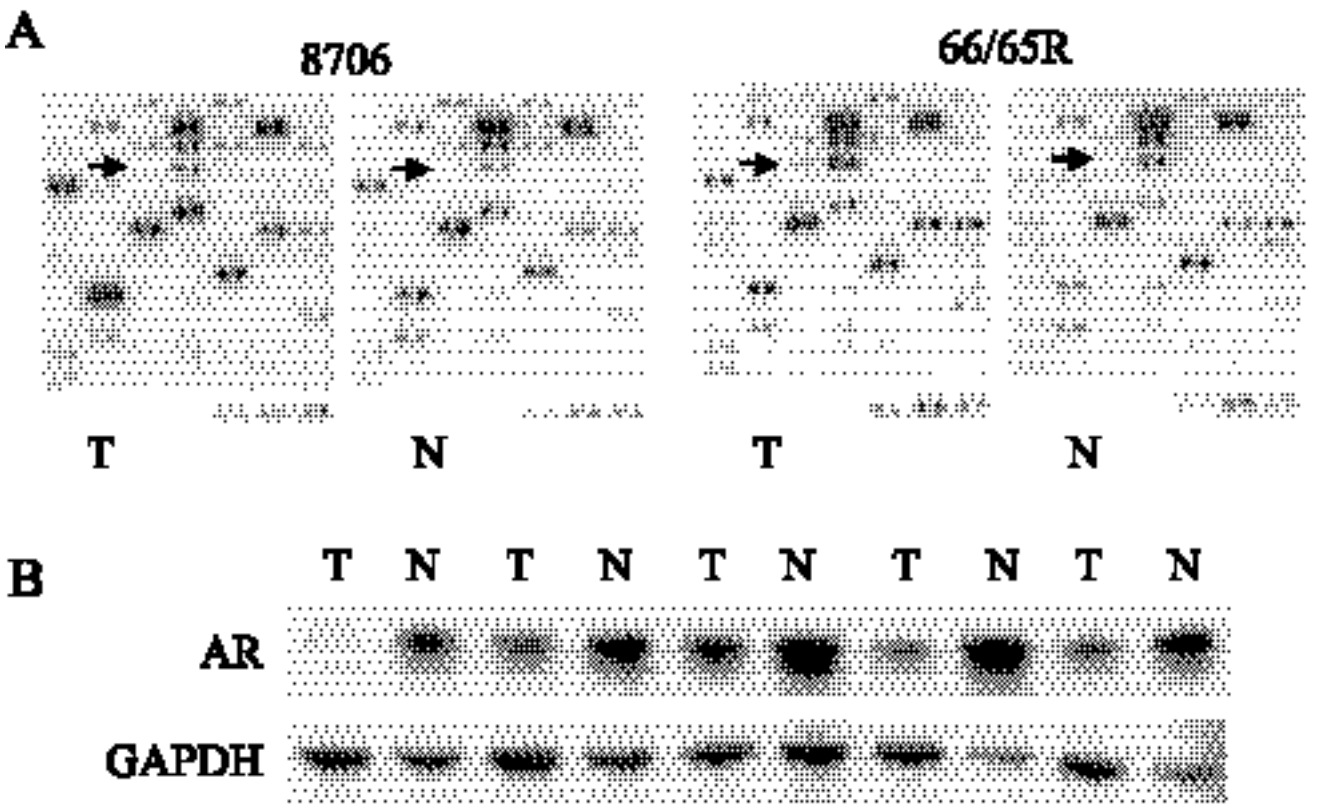

Figure 3. *Differential expression of AR. (A) cDNA expression array hybridization with the squamous cell carcinoma 8706 and its matching normal tissue and the adenocarcinoma 94-11-C066R (66R) and its matching normal tissue 94-11-C065R (65R). (B) RT-PCR confirmation. The first two pairs of tumor/normal tissues from the left are squamous cell carcinomas of the lung. The remaining three pairs of tumor/normal tissues are lung adenocarcinomas.*

8% polyacrylamide gels with urea and exposed to Molecular Dynamics Phosphorimager screens for analysis. Densitometry was performed by ImageQuant software.

Results

The 588 human cDNAs immobilized on each Atlas human cDNA expression array consisted of 200 to 600 base pair fragments amplified from mRNA regions without polyA tails, highly homologous sequences, or base sequence repeats. This ensured that nonspecific background and cross hybridization of closely related gene family members was minimized. The low background levels acquired from use of primers specific for the genes immobilized on the nylon membrane allowed for the detection of mRNAs as rare as 10 to 20 copies per cell [26]. The cDNA spot signal intensity was compared to three housekeeping gene spot signal intensities (GAPDH, β-actin, and 60S ribosomal RNA gene) to determine the relative abundance of target cDNA in the hybridized sample.

Six different sets of cDNAs were immobilized on cDNA expression arrays at approximately 10 ng per spot including: 1) oncogenes, tumor suppressor genes, and cell cycle regulators; 2) ion channel and transport, stress response, and modulators, effectors, and intracellular transducer genes; 3) genes involved in apoptosis, DNA synthesis, repair and recombination; 4) transcription factors and DNA binding proteins; 5) receptors, cell surface antigens, and cell adhesion molecules; 6) growth factors, cytokines, chemokines, hormones, interleukins, and interferons [26].

Using the Altas human cDNA expression array, four sets of hybridizations were performed with reverse-transcribed polyA$^+$ RNA of two squamous cell carcinomas and their matching normal tissues (patients 8611 and 8706) and two adenocarcinomas and their matching normal tissues (patients 8641 and 94-11-C066/65R). Representative examples of the pathologic features of an adenocarcinoma and its paired normal alveoli as well as a squamous cell carcinoma and its paired normal bronchiole are shown in Figure 1.

Of the 588 cDNAs present on the cDNA expression array, an average of 262 spots (45%) were detected after hybridization. After normalization to housekeeping genes GAPDH, β-actin, and 60S ribosomal protein L13A, sequences that were differentially expressed by at least two-fold between tumor and normal tissues were selected for further confirmation. Because each housekeeping gene standardized the cDNA spot intensities to different relative levels, only those cDNAs that were differentially expressed by at least two-fold when normalized to each of these housekeeping genes were examined further. The two-fold expression differences also needed to be detected in at least two of the

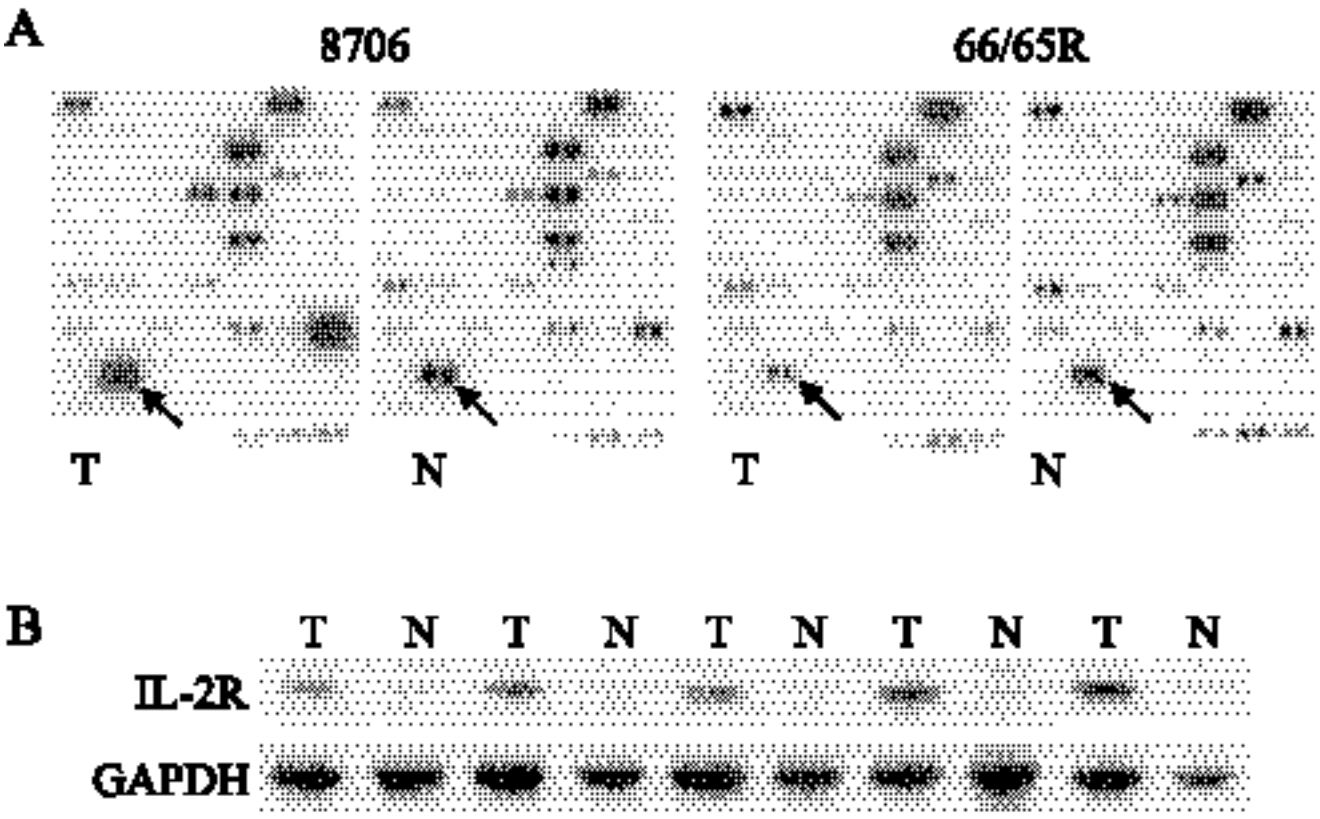

Figure 4. *Differential expression of IL-2Rα. (A) cDNA expression array hybridization with the squamous cell carcinoma 8706 and its matching normal tissue and the adenocarcinoma 94-11-C066R (66R) and its matching normal tissue 94-11-C065R (65R). (B) RT-PCR confirmation. The first two pairs of tumor/normal tissues from the left are squamous cell carcinomas of the lung. The remaining three pairs of tumor/normal tissues are lung adenocarcinomas.*

four individual hybridizations that were performed, with two expression differences occurring in the same lung cancer subtype. Forty-five genes (17%) were differentially expressed in the cDNA expression array screening and selected for reverse-transcriptase polymerase chain reaction (RT-PCR) confirmation. Of these, 22 were overexpressed and 23 were underexpressed in the tumor tissues compared to their normal counterparts. The initial RT-PCR analysis was performed using seven pairs of matching normal/tumor tissues (three squamous cell carcinomas and four adenocarcinomas). Only the genes that displayed differential expression in >50% of the squamous cell carcinomas and/or adenocarcinomas were reanalyzed using the second set of seven matching normal/tumor tissues (four squamous cell carcinomas and three adenocarcinomas). Final analysis included 26 of the initial 45 genes (58%) that were differentially expressed by cDNA expression array analysis.

Of the 26 genes, 14 genes were overexpressed and 12 genes were underexpressed in the tumor tissues compared to their corresponding normal tissues (Table 2). Most of the differentially expressed genes were detected in both squamous cell carcinomas and adenocarcinomas. Eight genes were overexpressed or underexpressed in at least 70% of adenocarcinomas and squamous cell carcinomas. Of these, two were overexpressed (CD27L antigen receptor and ephrin A3) whereas six were underexpressed (*c-kit*, protein tyrosine kinase receptor tyro3, *c-fgr* proto-oncogene, TR3 orphan receptor, amphiregulin (AR), and macrophage inflammatory protein 2 alpha). However, some expression differences were more common in adenocarcinomas (*c-jun*, ephrin type A receptor 1, and interleukin 2 receptor alpha [IL2Rα]). Differential expression of intercellular adhesion molecule 1 (ICAM1) was more frequently detected in squamous cell carcinomas. The ras-related gene, *rab2*, was only differentially expressed in adenocarcinomas. Some genes were overexpressed in one NSCLC subtype but were variable or underexpressed in the other subtype. *Cdc25A* was overexpressed by at least two-fold in some squamous cell carcinomas but underexpressed in some adenocarcinomas compared to their respective normal tissues. Caspase 8 and insulin receptor were overexpressed in most adenocarcinomas but underexpressed in squamous cell carcinomas. Because sample sizes were small, more NSCLC subtypes need to be analyzed to substantiate these tendencies. Figures 2 – 4 show the representative results of differential expression of *c-jun*, AR, and IL2Rα. Further verification of differential gene expression in either squamous cell carcinomas or adenocarcinomas by Northern blot analysis could not be performed due to the limited amount of microdissected tumor tissues available for the present study.

Discussion

In this study, we compared the gene expression patterns of two subtypes of human NSCLC, adenocarcinomas and squamous cell carcinomas, to their matching normal lung tissues using Atlas human cDNA expression arrays. The use of this limited cDNA microarray with known genes, which were potentially related to cancer, certainly enriched for genes that might appear to be related directly to the cancer process. Approximately half of the 588 spotted cDNAs were detected in these normal and tumor tissues. Forty-five of the 262 cDNAs were differentially expressed according to the parameters set at the beginning of the study and were selected for further RT-PCR confirmation. Twenty-six genes including oncogenes, cell cycle regulators, intracellular signal transducers, apoptotic genes, cell receptors and adhesion molecules, and growth factors were differentially expressed in adenocarcinomas and/or squamous cell carcinomas compared to normal lung tissues obtained from the same patient. We found eight genes that were reproducibly (>70% of samples) altered in both adenocarcinomas and squamous cell carcinomas when compared with their normal controls. In general, the expression of genes identified in both adenocarcinomas and squamous cell carcinomas were routinely increased or decreased by more than two-fold.

Proto-oncogenes

Several members of this group were routinely altered in expression in both adenocarcinomas and squamous cell carcinomas. Interestingly, most of the genes with aberrant expression had decreased expression relative to their own controls including *c-kit*, *c-fgr*, *c-src*, and *c-jun*. *c-Kit* encodes a transmembrane tyrosine kinase receptor. It functions in cell survival, proliferation, migration, and differentiation [27]. The *c-kit* and stem cell factor (SCF) autocrine loop in SCLC may mediate chemotaxis and/or provide a growth advantage in these tumors [28,29]. Chemotactic and chemokinetic mobility was detected in lung cancer cell lines expressing *c-kit* and SCF [30]. Tonary et al. reported that *c-kit* expression was decreased in advanced stages of ovarian cancer and was associated with decreased survival [31].

Non–receptor tyrosine kinase, *c-src*, has been implicated in the tumorigenesis of multiple human cancers. Reported alterations involved both increases and decreases in enzymatic activity, gene copy number, and protein levels [32]. In lung cancer, elevated *c-src* expression was found in 60% of tumors of different histologic types, including adenocarcinomas and squamous cell carcinomas, compared to normal bronchial tissue [33,34]. Budde et al. reported high *c-src* activity for NSCLC cell lines but low activity in SCLC cell lines [35]. In our study, *c-src* levels were elevated in the majority of squamous cell carcinomas but were decreased in three of seven adenocarcinomas. This may suggest different functional roles for this proto-oncogene in these NSCLC subtypes.

c-Jun is a tightly regulated component of the activator protein-1 transcription factor complex. It is involved in cell proliferation and survival but can also induce apoptosis, growth arrest, and differentiation [36]. Expression analysis of *c-jun* in lung cancer has yielded conflicting results. Koomagi et al. reported elevated levels of *c-jun* in tumor

tissues of lung cancer patients compared to adjacent normal tissues [37]. However, in an additional study comparing 101 NSCLC specimens to adjacent normal lung tissue, 72% of the normal tissues expressed higher levels of c-jun [38]. In light of these observations, additional studies will need to be performed to define the role of c-kit, c-fgr, c-src, and c-jun in tumorigenesis.

Growth Factors and Related Genes

Several growth factors and related genes were also expressed differentially in the NSCLC subtypes compared to normal lung tissues. Hepatocyte growth factor (HGF) is a potent inducer of cell proliferation, motility, and morphogenesis [39]. A probable autocrine loop involving HGF and its receptor c-met exists in NSCLC [6,40]. HGF is secreted in an inactive form and must be proteolytically activated by factors such as HGF activator (HGFA) [41]. Although HGFA levels have not been examined in many tissues (including lung), significantly higher HGFA levels were reported in breast cancer tissue than in normal control tissues [39]. Increased HGFA expression was found in two of seven squamous cell carcinomas and five of seven adenocarcinomas.

Another differentially expressed gene in this study was AR. It is a strong growth promoter for breast, colon, lung, and ovarian epithelial cells and may function in autocrine and paracrine loops with the epidermal growth factor receptor (EGFR) to promote tumorigenesis [42,43]. However, it has also been reported to inhibit growth of carcinoma cell lines [44,45]. Both reduced (63%) and increased (11%) AR expression levels have been reported in NSCLC tumors compared to uninvolved lung tissue [46]. Most of the NSCLC samples in our study exhibited reduced AR expression compared to normal lung tissue. Only two of seven (28%) adenocarcinomas showed increased expression of AR compared to normal lung counterparts.

Intracellular Signal Transducers

Several modulators, effectors, and intracellular transducers showed increased expression in the human lung tumors. The receptor tyrosine kinase erythropoietin-producing hepatocellular (eph) family transduces signals to proteins involved in cytoskeletal organization, adhesion, migration, and proliferation [47,48] and may also be involved in angiogenesis [49-51]. Overexpression of eph receptors has been detected in gastric carcinomas, malignant melanomas, and hepatomas [52]. Overexpression of ephA1 receptor was able to transform NIH3T3 cells [48]. We found frequent overexpression of both ephA1 receptor and ephrin A3 ligand in both squamous cell carcinomas and adenocarcinomas compared to matching normal tissues.

LIM domain kinase (LIMK-1) is a serine/threonine kinase involved in the Rho GTPase signaling pathway that leads to actin cytoskeleton reorganization and the formation of lamellipodia [53-55]. Although LIMK-1 has not previously been implicated in cancer, it was overexpressed in two of seven squamous cell carcinomas and four of seven adenocarcinomas in this study.

Apoptotic Factors

Another component of neoplastic transformation is the prevention of apoptosis. Death receptor–dependent and –independent activation of caspase 8 leads to cleavage and activation of effector caspases 3, 6, and 7 and other intermediate apoptotic signaling proteins [56,57] that then initiate cell death. In neuroblastoma, caspase 8 was often inactivated by gene deletion and/or DNA methylation in cells with *N-myc* amplification [58]. However, in several other cancers including high-grade non-Hodgkins lymphoma, breast carcinoma, pancreatic carcinoma, and gallbladder carcinoma, caspase 8 gene expression was upregulated [59-62] and was strongly associated with the extent of apoptosis [58,60,62]. In this study, *caspase 8* was downregulated in several squamous cell carcinomas (3/7) but primarily upregulated in adenocarcinomas (3/7).

Using the rapid, high-throughput human Atlas cDNA expression array system, we examined the gene expression profiles of 14 NSCLCs and their matching normal tissues. Numerous cell surface receptors, proto-oncogenes, growth factors, and signal transduction molecules were differentially expressed in these NSCLC subtypes and may have significant roles in lung tumorigenesis. The divergent expression patterns of *c-src*, *caspase 8*, *cdc25A*, *rab2*, and *ICAM1* (see Table 2) suggest subtle but probable molecular differences between human lung adenocarcinomas and squamous cell carcinomas. These differences may ultimately play a role in determining the best treatments for these two NSCLC subtypes. Differential gene expression in tumor and normal lung tissues does not necessarily suggest tumor promoter or tumor inhibitory function. These differences may be a consequence of other biochemical changes that occur in a cell on its path to neoplastic transformation. Nevertheless, the changes in gene expression may serve as diagnostic or prognostic tumor biomarkers. Others may prove to be modulated by therapeutic or preventative agents and, thus, serve as surrogate endpoint biomarkers.

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