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ScienceDirect
Life Sciences 82 (2008) 772–779
Life Sciences
www.elsevier.com/locate/lifescie

Molecular profiling of genes in squamous cell lung carcinoma in Asian Indians

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Received 6 August 2007; accepted 18 January 2008

Abstract

Early metastasis and a poor five-year survival make lung cancer the leading cause of cancer related deaths worldwide. The clinical profile of lung cancer patients in India differs from the West as they present earlier, with squamous cell carcinoma being the commonest histological type. We compared gene expression profiles in primary lung squamous cell carcinoma (LSCC) and matched normal lung tissues in Asian Indians. Using suppression subtractive hybridization, two subtracted cDNA libraries containing differentially expressed genes in the tumors were constructed. Differential expression was confirmed by reverse Northern blot analysis. DNA of confirmed clones was sequenced and subjected to GenBank Blast searches. RNA expression levels were then analyzed by Northern blotting and validated by semiquantitative RT-PCR (in 10 cases of NSCLC). Seventeen differentially expressed gene cDNA fragments of LSCC were analyzed. The differentially expressed genes included those associated with cellular metabolism, cell-cycle, -structure, -adhesion, transcription, proliferation, apoptosis and signal transduction. The study provided first evidence that KIAA0767, a Death Inducing Protein, a novel p53 independent target of E2F1, and Geminin, an inhibitor of DNA replication are differentially expressed in LSCC. Identification of the differentially expressed genes in lung cancer in this study may serve as better molecular markers for early diagnosis and identifying novel intervention sites for anticancer therapy.

Keywords: Differential gene expression; Squamous cell lung carcinoma; Asian Indians

Introduction

Despite significant progress towards elucidation of human lung cancer tumorigenesis in the past two decades, it still remains the leading cause of cancer related deaths in most nations worldwide (Parkin et al., 2001). Early metastatic spread results in a dismal five-year survival of only 8–15% (Greenlee et al., 2000). In India, it is estimated that lung cancer constituted approximately 41,000 new cases in the year 2001 (National Cancer Registry Program, 2001). The clinical profile of lung cancer in India differs from the West, in that Indian patients present almost 15–20 years earlier than their western counterparts. Furthermore, Squamous Cell Carcinoma (SCC) is the commonest histological type in India compared to Adenocarcinoma (ADC) in the West (Jindal and Behera, 1990). The mortality from lung cancer has changed very little over the past few decades, which emphasizes the need for therapies based on a greater understanding of the molecular changes that underlie lung cancer (Sen et al., 2005). Although several genes have been reported and tested as diagnostic and prognostic markers for lung cancer, there is scope for improvement in this area (Sen et al., 2001).

A PCR based cDNA subtraction technique termed suppression subtractive hybridization (SSH) allows selective amplification of target cDNA while simultaneously suppressing non-target cDNA amplification. SSH has been successfully applied

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0024-3205/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.lfs.2008.01.009

as an efficient method to identify differentially expressed genes in cancer cells in comparison with their normal counterparts (Petersen et al., 2000; Shen et al., 2002; Bangur et al., 2002). Although cDNA microarray is increasingly applied for the parallel analysis of gene expression, SSH is still widely used since it enables the recovery of abundant as well as low copy number mRNA transcripts. SSH not only permits an efficient identification of tumor associated genes with known function, but also an unbiased isolation of novel sequences that are not yet available on the microchips. In this study, we compared gene expression profiles in primary, untreated, lung squamous cell carcinoma (LSCC) and matched normal lung tissues in Asian Indians.

Materials and methods

Patients and tissue sample collection

Twelve patients with primary NSCLC (11 SCC and 1 ADC) were operated at the All India Institute of Medical Sciences, New Delhi. Chemotherapy and/or radiotherapy were not applied before surgery for any of the patients. The histological diagnosis was re-confirmed by a pathologist for all the samples. The tumor tissue and its paired morphologically normal adjacent lung tissue (at least 10 cm away from tumor margin) were obtained immediately after resection from the patients and stored in RNA later™ (Ambion, Austin, TX, USA) at −70 °C.

Extraction of total RNA and mRNA

Total RNA was extracted from all the normal and tumor tissues using Tri-Reagent (Sigma, St. Louis, MO, USA). From these, mRNA was isolated for one stage II and one stage III patients using mRNA isolation kit (Roche, Germany) having streptavidin magnetic particles and oligo(dT) biotin labeled probes, following manufacturer's protocol. The quality and quantity of RNA/mRNA were determined using 1% agarose/denaturing formaldehyde gel electrophoresis and ultraviolet spectrophotometry. The RNA was stored in 70% ethanol at −70 °C deep freezer until use.

Construction of subtractive cDNA libraries

For the construction of subtractive cDNA libraries, Clontech PCR-Select™ cDNA subtraction kit and Advantage 2 Polymerase mix (BD Biosciences Clontech, Palo Alto, CA, USA) were used. 2 μg of poly A^+ RNA from the two normal and tumor tissues (SCC) were used as templates to synthesize single

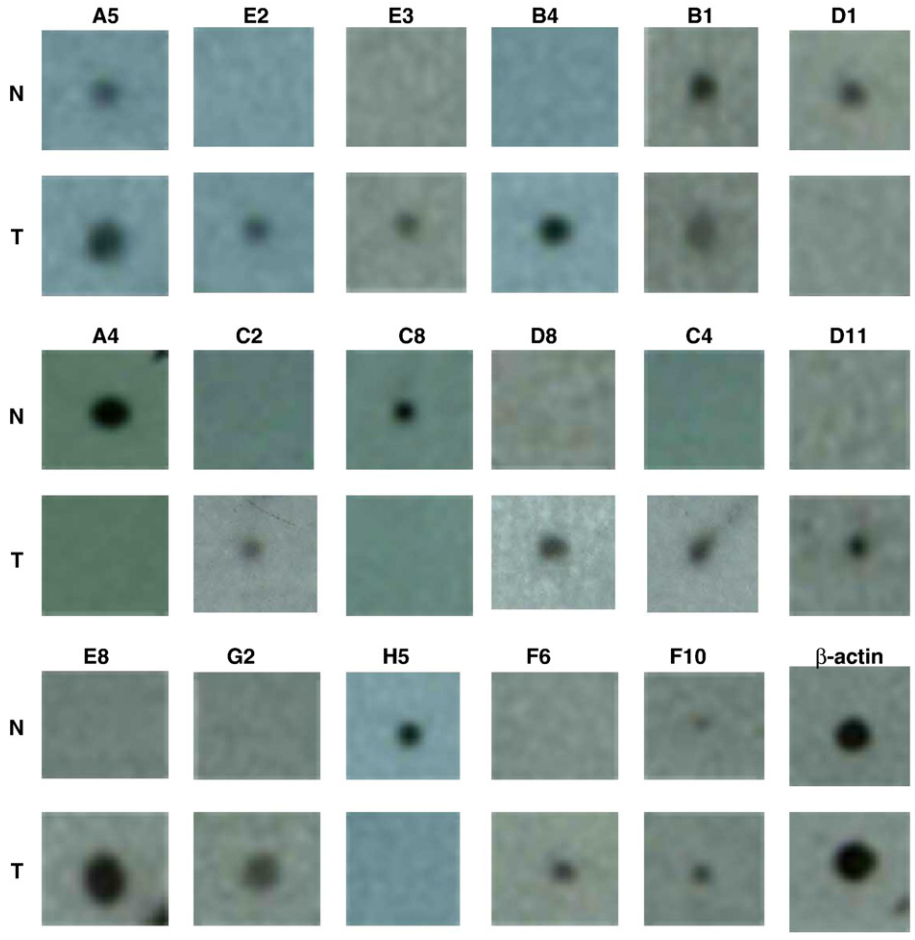

Fig. 1. Reverse Northern blot screening of differentially expressed cDNA fragments. After denaturation, plasmids containing the cloned cDNA fragments were blotted onto replicas of nylon membrane. Membranes were hybridized with ^{32}P labeled cDNA probes made from the RNAs of either normal (N) or tumor (T) lung tissue by reverse transcription. β-actin was used for normalization. Details of different clones are given in Table1.

stranded cDNA using Oligo(dT) primer and AMV-RT which were subsequently converted to double stranded cDNA (ds cDNA) according to manufacturer's protocol. One microgram each of prepared tester and driver ds cDNA was digested by Rsa I enzyme, the tester cDNA was separated into two parts and then ligated to adaptor 1 and adaptor 2R in separate ligation reaction mixtures (50 mM Tris–HCl, pH 7.8, 10 mM $MgCl_2$, 1 mM DTT, 1 mM ATP, 5% polyethylene glycol and 0.5 U T_4 DNA ligase) at 16 °C overnight. The reaction was stopped by EDTA/glycogen and ligase was inactivated by heating the samples at 72 °C for 5 min. For the first hybridization, an excess of driver cDNA (600 ng) was added to each tester cDNA (ligated with adaptor-1 or -2R; 20 ng each) in separate samples. After denaturation at 98 °C for 1.5 min, the first hybridization was performed in a hybridization buffer at 68 °C for 8 h. For the second hybridization, the two samples of the first hybridization were combined (without denaturation) at 68 °C overnight. Two PCR amplifications were performed for each subtraction. A primary PCR was used to selectively amplify the differentially expressed sequences with 1 μl of the diluted subtracted cDNA in a 25 μl

Table 1
Differentially expressed genes in normal and tumor human lung carcinoma (SCC) tissues in two subtractive libraries, L1 and L2

Clone ID	Gene description (% homology)	GenBank accession no.	Chromosomal localization and relevant gene ontology
A5	(Pancreatic tumor related protein) Human eukaryotic translation elongation factor 1-gamma (100%)	BC 031012	11q12 GO:0003746 GO:0005515
E2	Human cDNA clone (100%)	BC 073782	14q32 GO:0003823 GO:0005515
E3	(Hypothetical protein FLJ12892) Human coiled coil domain [CCDC14] containing 14 (99%)	NM 022757	3q 21 GO:0009055 GO:0005507
B4	Human ribosomal protein L30 (100%)	NM 000989	8q22 GO:0006412 GO:0003723
B1	Human 3-oxoacid CoA transferase (SCOT or OXCT) nuclear gene encoding mitochondrial protein (99%)	NM 000436	5p13 GO:0008152 GO:0016740
D1	Human ribosomal protein S7 (100%)	NM 001011	2p25 GO:0006412 GO:0003723
A4	Human trophoblast MHC class II suppressor mRNA (97%)	AF 508303	11q13
C2	Human in RNA for KIAA0767 protein, DIP (100%)	AB 018310	22q13 GO:0016984
C8	Human non-POU domain containing, octamer binding (NONO) mRNA (100%)	NM 007363	Xq13 GO:0006355 GO:0006281
D8	Human Calcitonin/calcitonin-related polypeptide, alpha (CALCA) gene (99%)	DQ 080435	11p15 GO:0051482 GO:0007267 GO:0016481
C4	Human collagen, type I, alpha I (COLIAI) (100%)	BC 036531	17q21–q22 GO:0005201 GO:0005515
D11	Human ATPase, H^+ transporting, lysosomal accessory protein (ATP 6AP1) (100%)	NM 001183	Xq28 GO:0045449 GO:0050677 GO:0008083
E8	Human, Geminin, DNA replication inhibitor (100%)	NM 015895	6p22 GO: 0000074 GO:0008156 GO:0007049
G2	Human CD9 Ag (p24) CD9 gene (99%)	AY 422198	12p13 GO:0007155 GO:0006928
H5	Human ribosomal protein L8 (RPL8) (98%)	BC 013104	8q24 GO:0006412 GO:0019843
F6	Human ribosomal protein S8 (RPS8) (100%)	X 67247	1p34 GO:0006412 GO:0003723
F10	Human trophoblast MHC class II suppressor mRNA (100%)	AF 508303	11q13

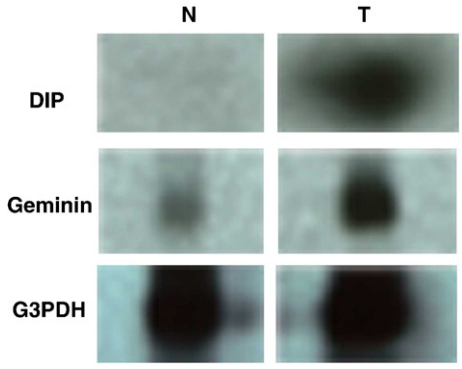

Fig. 2. Northern blot analyses of Death Inducing Protein (DIP) and Geminin. Normal (N) and tumor (T) lung tissues of the patients of the respective libraries (L1 and L2) were used. Equal amounts of total RNA of normal and tumor tissues were loaded and PCR-amplified, radiolabelled, double stranded DNA (using gene specific primers) was used as probes. Normalization was done by reprobing with G3PDH radiolabelled probe.

volume containing 400 nM of each primer, 0.2 mM dNTPs and 0.5 U of Advantage Klen-Taq Polymerase (BD Clontech, Palo Alto, CA, USA). The efficiency of cDNA subtraction was evaluated by PCR by comparing the abundance of glyceraldehyde-3-phosphate dehydrogenase (G3PDH), a housekeeping gene, before and after subtraction.

Products from the secondary PCR were inserted into pGEMT Easy Vector™ (Promega, Madison, WI, USA) and transformed into *E. coli* strain DH5α. The bacteria were plated on ampicillin containing agar plates that also contained β-X-Gal and IPTG. After overnight incubation, a total of 110 white colonies were randomly picked from the two subtractive libraries and glycerol stocks made and stored at −70 °C. Plasmid DNA was isolated from each of the colonies and the insert checked on 1.5% agarose gel after digestion with Eco RI.

Screening using reverse Northern blotting

Plasmid DNAs (1 μg) were denatured in 0.4 M NaOH at 100 °C for 10 min and blotted onto positively charged nylon membranes (Roche, Germany). The membranes were UV cross-linked and rinsed in 6× SSC before hybridization. cDNA probes were prepared from 20 μg of normal and tumor RNA by reverse transcription in a 20 μl reaction that consisted of 20 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 5 mM DTT, 50 pmoles oligo(dT), 40 U RNase inhibitor, 1 mM dNTPs without dCTP, 4 μM unlabelled dCTP, and 25–100 μCi (α-^{32}P) dCTP (3000 Ci/mM). After 5 min incubation at 65 °C, the samples were shifted to 37 °C and 200 U MMLV reverse transcriptase (Invitrogen, CA, USA) was added followed by continued incubation for 1 h. After reverse transcription, a quick spin column was used to remove unincorporated ^{32}P and activity checked on a scintillation counter ($5–10\times10^6$ cpm). Equal amounts of the cDNA probes from normal and tumor tissues were heat denatured and used to probe the duplicate blots. The membranes were exposed to Kodak X-ray film for 3–7 days before development. Normalization was achieved by β-actin PCR product on both blots. Densitometric scanning of duplicate blots using an Alphaimager scanning densitometer (Alpha Innotech, USA) allowed for the calculation of the ratio of the signal obtained with tumor to normal cDNA probes and normal cDNA probes.

A second round of reverse Northern blotting was done to confirm specific genes. All other parameters were the same, however, the cDNA probes of normal and tumor belonged to a different set of LSCC and did not belong to the respective libraries. Positive clones arising after this second reverse northern were sequenced (Fig. 1).

Sequencing the subtracted cDNA clones and bioinformatics analysis

DNA sequencing was performed by automated means using M13 primers at Sequencing facility, South Campus, University of Delhi (New Delhi, India) for the selected cDNA clones. Nucleic acid homology searches were performed using BLAST program (Table 1).

Northern blot analysis

To confirm differential expression of two of the sequences detected by reverse northern analysis, forward and reverse gene specific primers were designed against the coding region for each of the selected sequences and synthesized by Microsynth (Switzerland). cDNA for the two normal and tumor RNA was synthesized using oligo(dT) primer and MMLV-RT (Invitrogen, CA, USA). [α-^{32}P] dCTP (3000 Ci/mM) and gene specific primers were used to synthesize double stranded radiolabelled probe. Total RNA (20 μg) from normal and tumor tissues were subjected to electrophoresis on 1.5% agarose formaldehyde denaturing gels. Following electrophoresis, the RNA was transferred to positively charged nylon membrane (Roche, Germany) by capillary action using 20× SSC to facilitate transfer. RNA was cross-linked to the nylon membrane using UV cross-linker. The membranes were hybridized with the

Table 2
Parameters for RT-PCR analysis of G3PDH and differentially expressed genes in NSCLC

Gene	Primers	Product size (bp)	Annealing temperature (°C)
G3PDH	Forward: 5′tgcaccaccaactgcttagc3′ Reverse: 5′tttctagacggcaggtcagg3′	297	60
DIP[a]	Forward: 5′agatgcccacggactacatc3′ Reverse: 5′gctgagaatggtctcgaagg3′	260	60
Geminin	Forward: 5′gccttctgcatctggatctc3′ Reverse: 5′ctccgtttttctgccacttc3′	231	60

[a] = Death Inducing Protein.

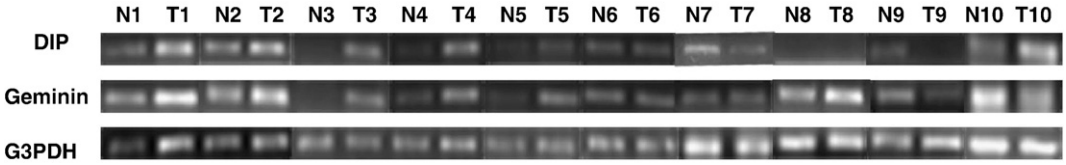

Fig. 3. Validation of subtractive hybridization data by RT-PCR. RT-PCR analyses were performed using 10 NSCLC tumor (T) tissues (9 SCC and 1 ADC) and matched normal (N) tissues. RT-PCR was used to amplify coding regions of DIP and Geminin. G3PDH, a housekeeping gene, amplified from the same samples was used for normalization.

probes for 16 h at 68 °C and later given high stringency washes with SSC and SDS and exposed to Kodak X-ray film for 1 week before development (Fig. 2).

Reverse transcription PCR

To confirm differential expression of the detected sequences, two significant sequences after performing homology search were chosen for RT-PCR analysis in 10 patients of lung cancer. 5′ and 3′ gene specific primers were designed against the coding region for each of the selected sequences and synthesized by Microsynth (Switzerland) (Table 2). To obtain semiquantitative results, the number of cycles of each RT-PCR, which was required for exponential DNA amplification, was first determined. RT-PCR was performed from total RNA isolated from the 10 lung cancer and adjoining normal tissues. Synthesis of the first strand was carried out using MMLV-RT (Invitrogen, CA, USA) and oligo (dT) primer. PCR amplification was carried out in a total volume of 25 µl using 2 µl cDNA, 10× PCR buffer, 10 mM dNTPs, 15 pmoles of each gene specific primer, 20 pmoles of G3PDH primers and 0.75 U *Taq* Polymerase (Biotools, Madrid, Spain). After 5 min of initial denaturation, 35 amplification cycles of 1 min at 94 °C, 1 min at 60 °C and 1 min at 72 °C were carried out followed by a 10 min elongation at 72 °C. The relative quantities of the specific PCR products were then determined by densitometric analysis and normalized with the density of the G3PDH profiles (Fig. 3).

Statistical analysis

Results of RT-PCR are expressed as normalized values. Statistical analyses were performed with Wilcoxon's Sign Rank Test using SPSS (Windows version 11.5). Values of $p<0.05$ were considered statistically significant.

Results

In an effort to identify genes that may be expressed differentially in human lung squamous cell carcinoma, we employed the SSH approach. Two SSH cDNA libraries were constructed one each with primary stage II and stage III lung squamous cell tumor and matched adjacent normal tissues to identify novel carcinogenic genes and possibly identify stage related genetic differences. They were considered to be good quality on determining the efficiency of subtraction. The libraries were named L1 and L2. A total of 110 subtractive cDNA clones (55 in L1 and 55 in L2) were obtained. Out of these, 17 genes showing significant differential expression (Fig. 1) were chosen for commercial DNA sequencing and nucleic acid homology search was performed using BLAST program. The differentially expressed genes included those associated with cellular metabolism, cell cycle, cell structure, cell adhesion, transcription, proliferation, apoptosis and signal transduction (Table 1).

Two genes, a mitochondrial Death Inducing Protein (DIP) and another, Geminin were randomly selected for further testing by Northern blotting (Fig. 2). There was positive correlation between Northern blot (Fig. 2) and the RT-PCR of the respective normal and tumor tissues of the L1 and L2 subtracted libraries (data not shown). To investigate whether the subtracted genes were indeed differentially expressed in lung cancer, confirmation and validation in 10 pairs of fresh primary NSCLC (9 SCC and 1 ADC) and their corresponding normal lung tissues were done by RT-PCR (Fig. 3). Out of 10 NSCLC cases in which expression of DIP was checked by RT-PCR, there was

Table 3
Semiquantitative RT-PCR analyses of two differentially expressed genes in NSCLC with clinico-pathological details

No.	Age (yrs)/sex	Pathological diagnosis	Histopath grading	Clinical staging	Smoking	DIP[h]	Geminin[i]
1	52/M[a]	SCC[b]	PD[d]	IIB	+[g]	10/20	10/5.2
2	50/M	ADC[c]	MD[e]	IIA	+	10/14.5	10/10
3	58/M	SCC	WD[f]	IIB	+	10/42	10/40
4	47/M	SCC	MD	IIIA	+	10/29.7	10/15.5
5	49/M	SCC	MD	IIB	+	10/5.5	10/6.9
6	54/M	SCC	MD	IIB	+	10/14.2	10/15
7	57/M	SCC	MD	IIIA	+	10/1.43	10/14
8	56/M	SCC	MD	IIB	+	No exp	10/13.3
9	62/M	SCC	MD	IIA	+	10/1	10/7.4
10	62/M	SCC	WD	IIB	+	10/14	10/7.6

[a] = Male; [b] = Squamous cell carcinoma; [c] = Adenocarcinoma; [d] = Poorly differentiated; [e] = Moderately differentiated; [f] = Well differentiated; [g] = Smoker; [h] = DIP expression (normalized values) Normal/Tumor; [i] = Geminin expression (normalized values) Normal/Tumor.

increased expression in the tumor in 60% (6/10) cases (ranging from 1.4 fold to 4 fold) ($p=0.014$) and decreased expression in tumors in 30% (3/10) cases (ranging from 1.8 fold to 10 fold) ($p=0.083$) and no expression (despite expression of house-keeping gene) in 1 case. Out of 10 NSCLC cases in which expression of Geminin was checked by RT-PCR, there was increased expression in tumor in 50% (5/10) cases (ranging from 1.3 fold to 4 fold) ($p=0.025$) and decreased expression in tumor in 40% (4/10) cases (ranging from 1.4 fold to 2 fold) ($p=0.046$) and same expression in normal and tumor tissue in 1 case (Table 3).

Discussion

Changes in gene expression affect the biological functions of all living cells. Cell growth, development, differentiation, cellular senescence and apoptotic cell death are some of the normal cell processes regulated by changes in the gene expression pattern. Dysregulated expression often results in pathological conditions such as cancer (Varmus, 1989). Using SSH, we compared gene expression profiles in primary lung squamous cell carcinoma (LSCC) and matched normal lung tissues in Asian Indians.

Some of the known genes recovered by us from Indian patients of lung cancer (LC) by SSH have previously been correlated with lung cancer and on comparison with published gene databases, genes like the eukaryotic translation elongation factor have been found to be expressed in American and Chinese populations (Bangur et al., 2002; McDoniels-Silvers et al., 2002; Liu et al., 2007), Calcitonin related (CALCA) gene in European populations (Amatschek et al., 2004), Collagen IAI gene in European populations (Amatschek et al., 2004), ion transporters resembling ATP 6AP1 in American and European populations (McDoniels-Silvers et al., 2002; Difilippantonio et al., 2003), NONO gene in Chinese populations (Liu et al., 2007), and ribosomal proteins in American and Chinese populations in different cancers (McDoniels-Silvers et al., 2002; Liu et al., 2007). This suggests a certain degree of overlap in genetic profiles of differentially expressed genes in different populations like Americans, Europeans, Chinese and Asian Indians. Our study not only supplements other studies analyzing differential gene expression in lung carcinoma in a different ethnic population and finds certain similarities between these populations but further identifies some new genes like DIP, Geminin, SCOT, CCDC14, trophoblast MHC class II suppressor, CD9 (p24), etc. in Asian Indians, which may not only have potential diagnostic, prognostic and therapeutic relevance but may also help in unravelling the puzzle of a different type of lung cancer predominating in this part of the world. In addition to the known genes, we have also identified a few genes that have as yet no functional annotation. To validate the clinical relevance of the up/down regulated genes identified by the SSH method, a subset of differentially expressed sequences was chosen on the basis of our areas of interest and further screened and validated by Northern blotting and RT-PCR.

One of the cDNA fragments upregulated in one subtractive library showed homology to the KIAA0767 gene product, termed Death Inducing Protein (DIP). KIAA genes comprise a family of novel large size (>4 kb) human cDNAs identified in the Kazusa cDNA sequencing project (HUGE), systematically designated KIAA plus a 4-digit number (Kikuno et al., 2002). DIP is a novel p53 independent target of the transcription factor E2F1 (Stanelle et al., 2005). E2F1 has unique and somewhat paradoxical activities. It promotes cell proliferation by stimulating expression of a number of genes that promote transition from G1 to S phase (Ishida et al., 2001). Concomitantly it also induces apoptosis through various inter-related pathways (Ginsberg, 2002). Our study provides an interesting insight into the incompletely understood regulatory mechanism of E2F1. The variability in differential expression in lung cancer (Table 3) can be explained by the fact that though DIP is reported to be pro-apoptotic (Stanelle et al., 2005), there is substantial evidence that E2F1 can contribute to tumorigenesis in vivo and vitro (Ishida et al., 2001; Pierce et al., 1998). Our results show a significant increase in DIP expression in tumors (6 out of 10 cases; $p=0.014$) while the decreased expression is not statistically significant (3 out of 10 cases; $p=0.083$).

A cDNA fragment upregulated in the tumor in the second subtractive library showed homology to Geminin, an inhibitor of DNA replication. It interacts with Cdt1p and prevents the recruitment of Mcm2-7p complex to the origin during S, G2 and early M phases of the cell cycle and thereby inhibits replication initiation on re-replication (McGarry and Kirschner, 1998; Wohlschlegel et al., 2000). In the normal cell cycle, however, Geminin is not present in G1 and thus does not impede the normal establishment of pre-RCs (pre-replicative complexes). Geminin expression begins as the origins are triggered at the G1–S transition and the protein level rises through the rest of the cell cycle to reach a maximum in mitosis. This inhibition of pre-RC assembly during the latter half of the cell cycle ensures that the same origin is not activated twice in the same cell cycle and thus acts to maintain genomic integrity by preventing abnormal re-replication of DNA (Diffley, 2001). Geminin has been found to be involved in the pathogenesis of breast, colon and renal cell carcinoma as an oncogene, although its exact role in tumor development remains to be determined (Montanari et al., 2005; Shetty et al., 2005; Bravou et al., 2005; Dudderidge et al., 2005). However, addition of Geminin to cell free DNA replication reactions derived from *Xenopus* egg extracts suppresses replication initiation and prevents cell cycle progression (McGarry and Kirschner, 1998; Wohlschlegel et al., 2000). It has been hypothesized that Geminin might be a classic tumor suppressor gene and its decreased expression might promote cell proliferation (Lygerou and Nurse, 2000). Our study reports its differential expression in lung carcinoma for the first time. Interestingly, there is increased expression in 50% tumors (5/10 cases) and decreased expression in 40% tumors (4/10 cases) with no change in expression in one case (Table 3). This underlines both the oncogenic as well as tumor suppressor functions of Geminin. The idea that Geminin might have distinct positive and negative functions with respect to cell cycle progression is not unprecedented and has been previously proposed for the Cdk inhibitor, p21 (LaBaer et al., 1997; Wohlschlegel et al., 2002).

Other interesting findings in this study were the differential expression of Elongation Factor 1 gamma (EF1γ), p24 (CD9 gene), COL1A1 and CALCA genes. EF1γ has been found to be

over-expressed in various gastro-intestinal tract malignancies and is useful in predicting tumor aggressiveness or their malignant potential (Mimori et al., 1996). Recent studies have demonstrated presence of p24 (CD9)/hsp27 in breast cancer as a factor predicting earlier relapse, response to therapy and duration of response (Seymour et al., 1990). COL1A1 has also been found to be differentially expressed in breast and lung carcinoma (Amatschek et al., 2004). The CALCA gene which codes for Calcitonin, was found to be differentially expressed in lung adenocarcinoma, glial tumors and has been associated with methylation induced gene silencing in various cancers (Amatschek et al., 2004; Widschwendter et al., 2004).

Some other mRNA for lesser known genes like the 3-Oxo acid CoA transferase (SCOT), Human NonO homologue, $p54^{nrb}$, ribosomal proteins, S7, S8, L8, trophoblast MHC class II suppressor, ATPase H^+ transporting lysosomal accessory protein and human coiled coil domain containing 14 (CCDC14) have also been found to have differential expression in this study.

Through the application of this technique for the first time to primary SCC patients in the Asian Indian population, we report the isolation of several novel genes of which the differential expression is likely to be of diagnostic, prognostic and therapeutic relevance and may also help in unravelling the puzzle of a different type of lung cancer predominating in this part of the world and perhaps throw up stage related genetic differences when validated in larger cohorts. This study also underlines the need for a new classification of lung cancer that includes genetic composition and gene expression profiles. We have provided detailed sequence information on transcriptional changes associated with lung carcinogenesis in Asian Indian population, many of them being described for the first time in lung cancer. The specific set of genes we recovered by cDNA subtraction provides a basis for identifying transcripts with diagnostic value with respect to primary SCC. In addition, studying the function of these genes and their biological pathways may lead to development of new therapeutic options. Further studies on DIP and Geminin on larger number of patients as well as functional characterization are also being undertaken. We believe that the differentially expressed genes found in this study may provide new insights/clues for further study on human lung carcinogenesis especially in the Asian Indian population.

Acknowledgements

This work was supported by the Department of Biotechnology (DBT), India. SS is grateful to Dr. Biplab Bose for valuable suggestions, Mr. Kulwant Singh Kapoor for helping in statistical analysis and Mr. Tirtho Dasgupta for helping with artwork (digital alignment of figures).

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