Differential disruption of cell cycle pathways in small cell and non-small cell lung cancer

BP Coe*1,3, WW Lockwood1,3, L Girard2, R Chari1, C MacAulay1, S Lam1, AF Gazdar2, JD Minna2 and WL Lam1

1Department of Cancer Genetics and Developmental Biology, British Columbia Cancer Research Center, 675 West 10th Avenue, Vancouver, BC, V5Z 1L3, Canada; 2Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, Dallas, Texas 75390-8593, USA

Lung cancer is the leading cause of cancer-related mortality in the world, with small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) comprising the two major cell types. Although these cell types can be distinguished readily at the histological level, knowledge of their underlying molecular differences is very limited. In this study, we compared 14 SCLC cell lines against 27 NSCLC cell lines using an integrated array comparative genomic hybridisation and gene expression profiling approach to identify subtype-specific disruptions. Using stringent criteria, we have identified 159 of the genes that are responsible for the different biology of these cell types. Sorting of these genes by their biological functions revealed the differential disruption of key components involved in cell cycle pathways. Our novel comparative combined genome and transcriptome analysis not only identified differentially altered genes, but also revealed that certain shared pathways are preferentially disrupted at different steps in these cell types. Small cell lung cancer exhibited increased expression of MRPS5, activation of Wnt pathway inhibitors, and upregulation of p38 MAPK activating genes, while NSCLC showed downregulation of CDKN2A, and upregulation of MAPK9 and EGFR. This information suggests that cell cycle upregulation in SCLC and NSCLC occurs through drastically different mechanisms, highlighting the need for differential molecular target selection in the treatment of these cancers.

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Lung cancer is the leading cause of cancer-related deaths worldwide (Parkin et al, 2005). The disease is classified into two major histological groups: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). Tobacco smoke is a major etiological factor, especially in SCLC. Small cell lung cancer comprises approximately 20% of all lung cancers and exhibits a neuroendocrine phenotype while NSCLC lacks these features and makes up the remaining 80% of cases. Small cell lung cancer exhibits a more aggressive phenotype that inevitably reoccurs after initial response to chemotherapy while the clinical outcome of NSCLC is often hard to determine (Zakowski, 2003; Kurup and Hanna, 2004; Stupp et al, 2004). Much of our current knowledge of these subtypes has been derived from a canonical set of cell lines derived from primary tumours (Phelps et al, 1996). These lines have been particularly crucial in the understanding of SCLC for which surgical resection is rarely performed (Rostad et al, 2004). The variation in the development and progression of SCLC and NSCLC may be a result of underlying differences in genetic alteration. Although histological classification can separate these two subtypes, previous studies using conventional genome scanning techniques such as loss of heterozygosity analysis and comparative genomic hybridisation (CGH) have shown that differences and similarities in genetic aberration exist between SCLC and NSCLC (Girard et al, 2000; Balsara and Testa, 2002). The limited resolutions of these methods have hampered the ability to identify discrete differences in genetic alterations, which are essential to understanding the biochemical deregulation that lead to the unique phenotypes of NSCLC and SCLC. Furthermore, the lack of a well-defined progenitor cell type for SCLC has presented a major challenge in establishing specific gene expression levels (Coe et al, 2006).

Owing to these limitations, it has become apparent that combining genomic and gene expression data will be essential for identifying new tumour suppressors and oncogenes (Henderson et al, 2003; Tonon et al, 2005). In addition, many genomewide platforms have proved useful in defining recurrent regions of alteration in lung cancer cells (Tonon et al, 2005; Zhao et al, 2005). With the development of whole-genome tiling path array comparative genomic hybridisation (aCGH), segmental copy number changes unique to each cell type can be defined at high resolution (Ishkanian et al, 2004). This technology allows the fine mapping of genomic alteration boundaries to within a single bacterial artificial chromosome (BAC) clone, identifying the precise genes potentially affected by a copy number alteration (CNA). As alterations at the DNA level are the initial events in cancer development, the gene expression changes that occur as a result of these alterations will be important in tumorigenesis.

To determine novel differences in CNA between the two lung cancer cell types, we profiled the genomes of 41 lung cancer cell lines (14 SCLC and 27 NSCLC) using the whole-genome tiling path.
array for CGH analysis. The integration of expression data for these regions verified our findings and identified the gene expression changes associated with CNA. Furthermore, comparing expression and copy number levels between NSCLC and SCLC without the requirement for normal expression levels circumvented a significant hurdle in the analysis of SCLC. Additionally, difference-based analysis compensated for random cell culturing artefacts, allowing insight into the clinical disease. Grouping the differentially altered genes by biological function revealed cellular pathways that may drive the pathological development of these cell types. The discovery of these genes affected by phenotype-specific CNA (PSCNA) may shed light on disease mechanisms and identify novel molecular targets for therapeutics and diagnostics.

METHODS AND MATERIALS

DNA samples

The 41 lung cancer cell lines described were established at the National Cancer Institute (NCI-H series) and at the Hamon Cancer Center (HCC series) except for SW-900 and SK-MES-1 (Fogh et al., 1977; Phelps et al., 1996). These cell lines have been deposited for distribution in the American Type Culture Collection (http://www.atcc.org). DNA was extracted from 27 NSCLC: 18 adenocarcinomas (H1395, H1648, H1819, H1993, H2009, H2087, H2122, H2347, HCC78, HCC193, HCC366, HCC461, HCC1195, HCC1833, HCC3255, HCC4006, HCC827 and HCC2279) and 9 squamous cell carcinomas (H157, HCC15, HCC2450, HCC95, H520, H226, SW 900, SK-MES-1 and H2170), and 14 SCLC cell lines: nine classical (H187, H378, H889, H1607, H1672, H2107, H2141, H2171, and HCC33) and five variant (H82, H289, H524, H526, and H841). The identity of all 41 cell lines were verified by fingerprinting using the Powerplex 1.2 system (Promega) which contains nine polymorphic markers.

Tiling path copy array comparative genomic hybridisation

Segmental copy number status of the 41 lung cancer cell genomes were deduced in array CGH experiments using sub-megabase resolution tiling-set (SMART) arrays. These arrays contain 97299 elements representing 33.433 BAC-derived amplified fragment pools spotted in triplicate on two aldehyde-coated glass slides (Ishkanian et al., 2004; Watson et al., 2004). Array hybridisation was performed as previously described (Coe et al., 2006; Garnis et al., 2006). Briefly, 200–400 ng of sample and a common reference male genomic DNA (Novagen, Mississauga, ON, Canada) were separately labelled by random priming in the presence of cyanine-5 dCTP or cyanine-3 dCTP (PerkinElmer, Woodbridge, ON, Canada), respectively. Labelled sample and reference DNA probes were combined and purified using ProbeQuant Sephadex G-50 Columns (Amersham, Baie d’Urfe, PQ, Canada). The probe mixture was precipitated in a solution containing 100 μg Cot-1 DNA (Invitrogen, Burlington, ON, Canada) with 0.1% volume 3 M sodium acetate and 2.5 × volume 100% ethanol. The DNA pellet was resuspended in 45 μl of hybridisation solution containing 80% DIG Easy hybridisation buffer (Roche, Laval, PQ, Canada), 100 μg sheared herring sperm DNA (Sigma-Aldrich, Oakville, ON, Canada), and 50 μg yeast tRNA (Calbiochem, San Diego, CA, USA) and denatured at 85°C for 10 min. Repetitive sequences were blocked at 45°C for 1 h before hybridisation. Probes were then added to array slides and placed in a pre-warmed hybridisation chamber (Telechem, Sunnyvale, CA, USA). After hybridisation for ~40 h at 45°C, arrays were washed five times for 5 min each in 0.1 × SSC, 0.1% SDS at room temperature in the dark with agitation followed by five rinses in 0.1 × SSC and dried by centrifugation.

Imaging and data analysis

Images of the hybridised arrays were captured through cyanine-3 and cyanine-5 channels using a charge-coupled device (CCD) scanner system (Applied Precision, Issaquah, WA, USA). Images were then analysed using SoftWoRx Tracker analysis software (Applied Precision, Issaquah, WA, USA). Spot signal ratio information was mapped to genomic coordinates and median normalised. Custom software called SeeGH was used to combine replicates and visualise all data as log2 ratio plots in SeeGH karyograms and exclude replicate data points which exceeded a standard deviation of 0.075 (Chi et al., 2004). In addition, genomic imbalances were identified using aCGH-Smooth which uses a genetic local search algorithm to identify breakpoints defining segmental DNA copy number changes by using a maximum likelihood estimation to optimise breakpoint location (Jong et al., 2004). As previously described, the Lambda and breakpoint per chromosome settings were set to 6.75 and 100, respectively (Jong et al., 2004; de Leeuw et al., 2004). The frequency of alteration for each BAC was then individually determined for each lung cancer cell type as described previously and plotted in SeeGH Frequency Plot to visualise areas of recurrent deletion and amplification (Coe et al., 2006). SeeGH software packages are available upon request at: http://www.ilmbox.ca./

Statistical analysis of array comparative genomic hybridisation alteration frequencies

Regions of differential copy number alteration between SCLC and NSCLC genomes were identified using a stringent multistep filtering process. The occurrence of copy number gain, loss, and retention at each locus was compared between SCLC and NSCLC data sets using Fishers exact test. Testing was performed using the R statistical computing environment on a 3 × 2 contingency table with a P-value threshold of 0.05. Loci for which the same cell type exhibited an increased frequency of both gain and loss when compared to the other were then excluded from these results in order to compensate for regions demonstrating higher levels of genomic instability but not true differential patterns of alteration. Finally, regions which passed the first two criteria and demonstrated alteration frequencies differing by at least 20% occurrence in either copy number loss or gain were selected for further analysis.

Affymetrix gene expression analysis

Affymetrix HG-U133A and HG-U133B hybridisations were performed as described in Henderson et al. (2005). RNA expression profiles were generated for 14 SCLC and 22 NSCLC cell lines, all of which are present in the array CGH data set (H187, H378, H889, H1607, H1672, H2107, H2141, H2171, H82, H289, H524, H526, H841, H1395, H157, H1648, H1819, H1993, H2009, H2087, H2122, H2347, H3255, HCC1195, HCC15, HCC1833, HCC193, HCC2279, HCC2450, HCC366, HCC4006, HCC461, HCC827, HCC95). Absolute expression values were log-transformed and scaled to a score between 0 and 100 using MAS 5.0 (Affymetrix, Santa Clara, CA, USA), and only probe sets demonstrating a present or marginal quality score in at least 50% of samples were considered for further analysis. Gene expression data for SCLC and NSCLC were then compared using the Mann–Whitney U test to identify genes that differed in expression between the two cell types with a P-value of at least 0.001. The resulting gene list was then filtered to select only those genes for which the expression change matched the direction predicted by the copy number analysis.

Real-time polymerase chain reaction

Real-time PCR validation of expression differences between NSCLC and SCLC was performed on key genes identified through combination of array CGH and Affymetrix gene expression...
profiling. Five micrograms of total RNA from each cell line profiled by Affymetrix microarrays was converted to cDNA using an ABI High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA, USA). A measure of 100 ng of cDNA was used for each real-time PCR reaction. TaqMan (Applied Biosystems, Foster City, CA, USA) gene expression assays: E2F2 (Hs00231667_m1), SOX11 (Hs00846583_s1), MAP3K4 (Hs00245958_m1), HSPH1 (Hs00198379_m1), B-actin (Hs99999903_m1), 18S rRNA (Hs99999901_s1) were performed using standard TaqMan reagents and protocols on a Biorad I-cycler (Biorad, Hercules, CA, USA). The ΔΔCt method was used for expression quantification using the average of the cycle thresholds for B-actin and 18s RNA to normalize gene expression levels between samples. Expression levels were compared between NSCLC and SCLC by a Mann–Whiney U test as performed for the Affymetrix microarray data.

Principal components analysis

The 243 Affymetrix probe sets deregulated as a result of copy number differences between SCLC and NSCLC were subjected to principal component analysis. Analysis of the samples was performed using the Statistics Toolbox (Version 5.1) of MATLAB (Version 7.1) (The MathWorks Inc., Natick, MA, USA).

RESULTS AND DISCUSSION

Copy number analysis of lung cancer cell genomes

To facilitate the high-resolution search for novel genetic alterations unique to each lung cancer cell type, we analysed 14 SCLC and 27 NSCLC cell lines with the SMRT CGH array. This array allows the accurate assessment of segmental DNA copy number changes at 32 433 overlapping genomic loci in a single experiment, producing copy number maps at 100 kbp resolution across the entire sequenced human genome (Ishkanian et al., 2004). After co-hybridising differentially labelled sample DNA and a male genomic DNA reference, fluorescence signal intensity ratios for each array element were determined and displayed as log₂ plots using SeeGH software. Genetic alterations were identified in all cell lines analysed. Figure 1 shows an example SeeGH karyogram for the SCLC cell line H1672. Upon visual analysis of this profile, areas of segmental gain and loss representing multiple levels of copy number change can be observed. For example, the telomeric end of chromosome arm 13q contains regions showing both single copy gain and high-level amplification (Figure 1). In addition to the multiple segmental alterations affecting the majority of chromosomes in this sample, discrete micro-amplifications and deletions are also detected such as those highlighted on chromosome arms 18q and 2q, respectively. These minute changes may have been missed by marker-based techniques and highlight the resolution of the tiling path array. Array CGH karyograms for all the cell lines are available online at http://www.bccrc.ca/cg/ArrayCGH_Group.html.

Frequency analysis

Regions of chromosomal alteration, key to the development of tumours, will be present in multiple samples. By aligning the profiles of multiple genomes, patterns of gain and loss are revealed and minimal regions that potentially contain tumour suppressor genes will be identified. The horizontal shift of each line to the left or right of 0 represents the measured Log₂ signal ratio from a competitive hybridisation with male genomic DNA. A decreased ratio represents a loss of copy number compared to the reference sample while an increased ratio represents an increase in copy number. Multiple levels of segmental copy number alteration as well as microalterations were readily detected (representative examples are highlighted in red and green). SeeGH karyograms for all cell lines analysed are available at http://www.bccrc.ca/cg/ArrayCGH_Group.html.
genes and oncogenes can be identified. Thus, after generating the whole-genome tiling path array CGH profiles of the lung cancer genomes, we then proceeded to identify recurrent regions of aberration within each cell type. To do this we employed a computer algorithm, aCGH-Smooth, to aid in the automated detection of regions of chromosomal gain and loss (Jong et al., 2004). The frequency of alteration of each genomic locus assayed was then calculated individually for the cell types and plotted using SeeGH Frequency Plot software as previously described (Coe et al., 2006). The data used to generate the frequency diagrams is present in Supplementary Material. The frequency plots and a detailed description of the recurrent regions of alteration specific to these SCLC and NSCLC cell lines have been reported (Coe et al., 2006; Garnis et al., 2006).

Genetic alterations unique to each cell type may contain genes responsible for the difference in disease development and clinical behaviour. To identify these regions, we overlaid the frequency plot diagrams of the SCLC and NSCLC samples and then compared the alteration frequencies in the two groups to determine regions that were statistically different by a $3 \times 2$ Fishers exact test and exclusion of regions which demonstrated increased gain and loss frequency for a single cell type (Figure 2). In this figure, areas indicated in green are more frequently altered in NSCLC while those in red are more frequently altered in SCLC. The yellow represents areas of overlap between the two frequency plots. Regions shaded in blue are those determined to be differentially altered in the cell types.

**Regions of similarity**

Among the regions that were not statistically different, there were some striking similarities (Figure 2). Consistent with previous reports, chromosome 3p loss was present in approximately 75% of both the NSCLC and SCLC samples (Balsara and Testa, 2002). This is consistent with previous results demonstrating that the deletion of putative tumour suppressor genes (TSGs), such as FHIT and RASSF1, contained on this chromosome arm are important genetic events in the development of lung cancers (Zabarovsky et al., 2002). Likewise, copy number loss of chromosome arm 4q was evident in $\sim 50\%$ of samples in each cell type mirroring results observed using conventional CGH (Petersen et al., 1997a, b)(Figure 2).

The NSCLC and SCLC cell lines also showed similar frequency of copy number gain on chromosomes arm 5p as well as at chromosome bands 7p22.3 and 11q13.1–11q14.1. Over-representation of the entire 5p arm was a recurrent event in both cell types with the telomeric end of 5p15.33 showing the greatest amount of change. This region contains the telomerase reverse transcriptase (hTERT) gene which has been implicated in cell immortalisation in numerous cancers (Tomoda et al., 2002; Ramirez et al., 2004). Gain of the 11q13.1–11q14.1 region was present in $>50\%$ of the lung cancer cell lines with the highest degree of concordance at 11q13.3 (Figure 2). Cyclin D1, which is involved in the inactivation of the retinoblastoma protein and progression of the cell cycle through the G1–S phase, is located at this loci (Muller et al., 1994). This finding supports the theory that amplification of this gene is an important event in tumorigenesis (Fu et al., 2004). The gain of 7p22 was particularly interesting as it was the most common copy number aberration in both cell types. The minimal common alteration within this amplified area in the SCLC cell lines contains only one gene, MAD1L1 (validated by Coe et al., 2006). Although this is a checkpoint gene involved in growth inhibition, its gain has been reported in other cancers (Jin et al., 1999; Tsukasaki et al., 2001; de Leeuw et al., 2004). The high frequency of MAD1L1

![Figure 2](image-url)  
**Figure 2** Copy number alterations in SCLC and NSCLC. Alteration frequencies for SCLC (green) and NSCLC (red) are displayed as bar plots adjacent to chromosomal ideograms. Bars extending to the right of each chromosome represent the frequency of copy number gain; conversely, bars extending to the left represent the frequency of copy number loss. Yellow regions represent overlapping portions of the SCLC and NSCLC alteration frequencies. Blue bars indicate regions demonstrating significantly different alteration frequencies. Vertical brown lines on the left of each frequency diagram indicate regions selected for further analysis.
Figure 3  Differential expression as a result of copy number alteration. Affymetrix log-transformed absolute expression data for the 243 probe sets exhibiting strong differential expression between SCLC and NSCLC associated with copy number differences are displayed. High-level expression is indicated by white/yellow while blue/black indicates progressively lower levels of expression. The SCLC samples are indicated by green highlighting of each column, while NSCLC samples are indicated by red highlighting. Each probe set is sorted according to its chromosomal position and cell lines are sorted alphabetically, according to their cell type. Probe set with annotated gene IDs are labelled with their RefSeq name while probe sets with less reliable mapping are indicated by their probe ID alone. Average expression values were calculated for genes with multiple Affymetrix probe sets, which passed the filtering conditions. These are indicated in blue text (The number of probe sets averaged is indicated in brackets). The primary genomic alteration observed for both SCLC and NSCLC are indicated to the right of each set of expression values (G = ‘gain’, L = ‘loss’, no value = ‘gained and lost’ or ‘no change’).
amplification in the NSCLC samples as well suggests that this gene may play an essential role in the development of lung cancers (Garnis et al., 2006).

It is noteworthy that a subset of the genomic similarities between the SCLC and NSCLC cell lines could be the result of adaptation to culturing conditions. Owing to this, the greatest insight into the biology of the clinical disease will be attainable through analysis of differences (rather than their similarities) in genomic alterations and gene regulation.

Regions of difference

Through our analysis, numerous regions throughout the genome were determined to be differentially altered between the SCLC and NSCLC samples. This difference-based approach compensates for random cell culturing artefacts and should identify the regions most strongly linked to clinical disease. These regions ranged in size from whole chromosomes (chromosome 21) to discrete peaks, kilobases in size (3q27.1). Using our stringent, multistep criteria (Fisher’s exact test followed by additional thresholding), we detected several regions that differed strongly in their alteration status between the cell types, we refer to these as phenotype-specific copy number alterations (PSCNAs). These included 1p36.33–1p34.2,
Identification of genes differentially expressed between small cell lung cancer and non-small cell lung cancer caused by phenotype-specific copy number alteration

Validation of the genomic differences identified between SCLC and NSCLC cell lines was performed by assessment of their impact on the gene expression level. This is achieved by integrating Affymetrix expression profiling data with the array CGH data as a consequence of the PSCNAs. To validate these CNAs and identify genes within these regions responsible for the different cell phenotypes, gene expression analyses were required.

| Gene symbol | Gene name | Locus | Regulation |
|-------------|-----------|-------|------------|
| STMN1       | Stathmin 1| 1p36.11 | SCLC +     |
| E2F2        | E2F transfection factor 2 | 1p36.12 | SCLC +     |
| ZNF151 (MIZ1)| Zinc-finger protein 151 (Myc-interacting zinc-finger protein) | 1p36.13 | SCLC +     |
| PROM2 (RIZ1)| PR domain-containing protein 3 (Rb protein-binding zinc-finger protein) | 1p36.2  | SCLC +     |
| ID2         | Inhibitor of DNA binding 2 | 2p21.35 | SCLC +     |
| SOX1       | SRY-related HMG-Box gene 1 | 2p25.5  | SCLC +     |
| MAPK9 (JNK2)| Mitogen-activated protein kinase 9 (C-JUN kinase 2) | 3q25.3  | NSCLC +    |
| MAP3K4      | Mitogen-activated protein kinase kinase 4 | 6q26   | SCLC +     |
| EGFR        | Epidermal growth factor receptor | 7p1.2  | NSCLC +    |
| CENID2A (p16INK4A)| Cyclin-dependent kinase inhibitor 2A | 9p2.13 | NSCLC -    |
| KNTC1       | Kinetochore-associated protein 1 | 12q24.31 | SCLC +     |
| HMG14B1     | High mobility group Box 1 (Aphorinam) | 13q12.3 | NSCLC –    |
| HSPH1       | Heat shock 105 kD | 13q12.3 | NSCLC –    |
| ING1 (p33ING1)| Inhibitor of growth family member 1 | 1q34    | SCLC +     |
| JAZF1 (SUZ12)| Joined to JAZF1 (Suppressor of ZESTE 12) | 17q11.2 | SCLC +     |
| NLRK        | Nemo-like kinase | 17q11.2 | SCLC +     |
| SWAD4       | Mothers against decapentaplegic homolog 4 | 18q2.1  | SCLC +     |
| CCDC5       | Coiled-coil domain containing 5 | 18q2.1  | SCLC +     |
| TCFC        | Transcription factor 4 | 18q2.12 | SCLC +     |
| JUNB        | Oncogene jun-B | 19p13.13 | SCLC +     |
| TIMM1       | T-cell lymphoma invasion and metastasis 1 | 21q22.11 | SCLC +    |
| DSCAM       | Down's syndrome cell adhesion molecule | 21q22.2 | SCLC +     |

SCLC = small cell lung cancer; NSCLC = non-small cell lung cancer; + = increased expression in the indicated cell type; – = decreased expression in the indicated cell type.

Figure 5 Differential targets of copy number-induced expression changes in key biochemical pathways between SCLC and NSCLC. Strong PSCNA-induced expression differences were identified between SCLC and NSCLC in several key pro-proliferative pathways. Genes with increased expression in SCLC when compared to NSCLC are indicated in green, while genes with increased expression in NSCLC are indicated in red. Genes exhibiting a tumour suppressor like pattern of reduced expression as a result of frequent copy number loss in NSCLC are indicated in yellow. Genes added to the pathways for context but for which no expression differences were detected, are indicated in grey. Critical pathway nodes validated by real-time PCR are indicated with a *.
expression in SCLC. This gene encodes an ABC transporter known to clear various chemotherapeutics from the cytoplasm and increased expression in lung cancer has been associated with exposure to platinum drugs (Oguri et al., 2000). Furthermore, another study has correlated MRPS expression to cisplatin chemoresistant lung cancer cell lines (Weaver et al., 2005). This result suggests a possible mechanism of enhanced chemotherapeutic resistance for the SCLC cells.

Biological pathways differentially altered in small cell lung cancer and non-small cell lung cancer

Further analysis of the differentially expressed genes revealed that a strikingly high number of genes are present in a small set of interconnected pathways. The presence of multiple genes affected by PSCNA in the MAPK and EGFR pathways lead us to examine the known interactors for each of these genes to elucidate a biochemical differentiation between SCLC and NSCLC cells. The results of this analysis are displayed in Figure 5. Twenty-two of the genes differentially altered between SCLC and NSCLC are components of the cell cycle, EGFR, MAPK, p38MAPK, and WNT pathways (Table 1). Four genes (E2F2, SOX11, MAP3K4, and HSPH1), which represent critical nodes in these pathways, were further examined by real-time PCR validating differential expression between SCLC and NSCLC. Pathway information was derived from the Signal Transduction Knowledge Environment (stke. sciencemag.org), the Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg), and the following references: Ishitani et al., 1999; Lundberg and Weinberg, 1999; Sakamuro and Prendergast, 1999; Taguchi et al., 2000; Shaulian and Karin, 2001; Zebedee and Hará, 2001; Hyodo-Miura et al., 2002; Schneider et al., 2002; Yamagishi et al., 2002; Bracken et al., 2003; Polager and Ginsberg, 2003; Williams et al., 2003; Wu et al., 2003; Campos et al., 2004; Einarson et al., 2004; Li and Guan, 2004; Rubin and Atweh, 2004; Wada and Penninger, 2004; Sasahira et al., 2005. Of particular interest was a strong increase in the expression of WNT inhibitors in SCLC cells, namely NLK, SOX11, and TCF4. This remarkable result demonstrates that the WNT pathway may not be a significant player in SCLC.

Additionally we detected a strong difference in the regulatory components of the p38MAPK pathway with the reduced expression of two p38 MAPK activating genes in NSCLC (HMGB1, HSPH1) and contrasting overexpression of two p38 MAPK activating genes in SCLC (MAP3K4, DSCAM). We also observed strong PSCNA-related overexpression of several members of the MAPK and cell cycle pathways in both cell types, albeit through different components. In the NSCLC samples, we observed segmental loss and downregulation of the cell cycle inhibitor CDKN2A as well as copy number gain and upregulation of MAPK9 and EGFR when compared to SCLC. In contrast, the SCLC cells demonstrate comparatively higher expression of many pro-proliferative genes; these are detailed in Figure 5. Interestingly, several genes with cell cycle inhibitory functions exhibited PSCNA-induced overexpression in SCLC. Owing to likely antagonism of these genes by the many upregulated cell-cycle-activating genes, it is possible that they perform a novel role secondary to their primary functions in cell cycle regulation. These differential patterns of oncogenic disruption to cell cycle pathways highlight the need to examine cell type-specific targets for therapeutic pathway intervention. For example, although a recent study has shown that EGFR is expressed at low levels in SCLC, (Tanno et al., 2004) our results indicate that the pathway is being activated by overexpression of multiple downstream components, potentially bypassing benefits that may be derived from EGFR-targeted therapy.

CONCLUSIONS

Whole-genome array CGH in conjunction with global expression profiling analysis has allowed the identification of genes deregulated as a result of PSCNA between SCLC and NSCLC cells. The 159 genes revealed as having strongly divergent expression patterns as a result of copy number alterations identified a remarkable pattern of gene deregulation in several key biological pathways. Cell cycle upregulation in SCLC and NSCLC occurs through drastically different targets, suggesting a need for differential therapeutic target selection. Additionally the WNT pathway, which has recently received much attention for its involvement in NSCLC, appears to be strongly downregulated in SCLC through PSCNA-induced overexpression of inhibitory genes. This work represents the first comprehensive search for the causative genetic alterations distinguishing SCLC and NSCLC by integrating whole-genome expression and copy number analysis platforms.

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