Genomic and gene expression profiling of minute alterations of chromosome arm 1p in small-cell lung carcinoma cells

L-J Henderson,1,2 BP Coe,1,2 EHL Lee,1,2 L Girard,3 AF Gazdar,3 JD Minna,3 S Lam,1 C MacAulay1 and WL Lam1,2

1British Columbia Cancer Research Centre, 675 West 10th Avenue, Vancouver, BC, Canada V5Z 1L3; 2Department of Pathology and Laboratory Medicine, University of British Columbia, G227-2211 Wesbrook Mall, Vancouver, BC, Canada V6T 2B5; 3Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, 6000 Harry Hines Blvd., Dallas, TX 75390, USA

Genetic alterations occurring on human chromosome arm 1p are common in many types of cancer including lung, breast, neuroblastoma, pheochromocytoma, and colorectal. The identification of tumour suppressors and oncogenes on this arm has been limited by the low resolution of current technologies for fine mapping. In order to identify genetic alterations on 1p in small-cell lung carcinoma, we developed a new resource for fine mapping segmental DNA copy number alterations. We have constructed an array of 642 ordered and fingerprint-verified bacterial artificial chromosome (BAC) array comparative genomic hybridisation (CGH) (Pinkel et al., 1998). This technique has been applied to map alterations across the genome at 1 megabase (Mb) resolution (Snijders et al., 2001; Greshock et al., 2002), at higher resolution for specific chromosomal regions and chromosome arms in several tumour types (Buckley et al., 2002; Garnis et al., 2003, 2004a), and, most recently, the whole human genome (Ishkanian et al., 2004).

Greater than 42 000 new cases of small-cell lung cancer (SCLC) are diagnosed annually in the United States, representing approximately 20% of all new lung cancer cases. Median survival time is 12 – 36 months and the majority of patients eventually die from the disease (Minna et al., 2003). Advancing our understanding of the molecular characteristics of SCLC may lead to the ability to better diagnose and treat the disease. One approach to this is the detection of regions of genetic alteration in the tumour genome in order to identify the genes that are causal to the disease. Identification of novel genetic changes correlated to specific human malignancies has typically been a tedious and laborious process. Recently, high-throughput and high-resolution detection of such alterations has been made possible through the use of bacterial artificial chromosome (BAC) array comparative genomic hybridisation (CGH) (Pinkel et al., 1998). This technique has been applied to map alterations across the genome at 1 megabase (Mb) resolution (Snijders et al., 2001; Greshock et al., 2004), at higher resolution for specific chromosomal regions and chromosome arms in several tumour types (Buckley et al., 2002; Garnis et al., 2003, 2004a), and, most recently, the whole human genome (Ishkanian et al., 2004).

Approximately 120 Mb of DNA span human chromosome arm 1p. Multiple regions on this chromosome arm have been implicated in SCLC and non-small-cell lung cancer (NSCLC) (Girard et al., 2000; Nomoto et al., 2000; Chizhikov et al., 2001; Ashman et al., 2002). Here, we describe the construction of a unique resource for analysing 1p. In all, 642 fingerprint-verified, physically mapped, overlapping BAC clones serve as target DNA for high-resolution BAC array CGH. Using this resource, we identified novel regions of DNA alteration in SCLC cells. We further defined the role of these alterations by employing Affymetrix oligonucleotide arrays and semiquantitative reverse transcriptase–polymerase chain reaction (RT–PCR) to analyse gene expression.

MATERIALS AND METHODS

BAC array construction and CGH

To design a minimal tiling path, BAC clones from the RPCI-11 library (Osoegawa et al., 2001) were selected from the FPC (Fingerprinted Contigs) database (International Human Genome Mapping Consortium, 2001). Wherever possible, sequenced and overlapping clones were chosen. Clones were obtained from glycerol stocks of the RPCI-11 library. DNA isolation and restriction enzyme digestion was performed as described previously (Marra et al., 1997). Each clone’s identity was verified by
comparing its HindIII DNA fingerprint to that found in the FPC database. The BAC DNA amplification, array printing, hybridisation, and analysis were performed as described previously (Garnis et al., 2004a). A log2 signal ratio of zero represents the most common copy number between the sample and normal reference DNA. The array was subjected to normal/normal hybridisation in order to eliminate clones that deviated from 0 by greater than +0.2 or less than −0.2. Clones with standard deviations among the triplicate spots greater than 0.075 were excluded from further analysis. A gain of copy number was defined as a log2 ratio of greater than 0.2, and a loss as a log2 ratio of less than −0.2. The average log2 ratio of two or more adjacent clones was required to be above the threshold in order for a region of alteration to be defined.

Cell lines
The lung cancer cell lines used were established at the National Cancer Institute (Phelps et al., 1996), with the exception of HCC33, which was generated at the Hamon Center for Therapeutic Oncology Research (Gazdar et al., 1998). NHBE (normal human bronchial epithelial cells) and SAEC (small airway epithelial cells) were obtained from Clonetics (San Diego, CA, USA). HBE2-KT, HBE3-KT, HBE4-KT, and HBE5-KT are normal human bronchial epithelial cell lines immortalised with CDK4 and hert (Ramirez et al., 2004). Culturing conditions and DNA isolation were described previously (Girard et al., 2000). RNA isolation was carried out using the RNeasy Midi kit (Qiagen, Valencia, CA, USA), together with in-column DNase I treatment.

Gene expression profiling using Affymetrix arrays
RNA fluorescent-labelling reaction and hybridisation were performed using the Affymetrix GeneChips HG-U133A and HG-U133B according to the manufacturer’s instructions (http://www.affymetrix.com). The arrays consist of 22,283 (HG-U133A) and 22,645 (HG-U133B) probe sets, which together amount to 24,698 unique genes based on Unigene build 163. Total RNA (5 µg) was used for each reaction. Microarray analysis was performed using Affymetrix Microarray Suite 5.0 and an in-house Visual Basic software MATRIX 1.24 (Girard et al., manuscript in preparation). Briefly, array data were median normalised and samples (or averages of samples) were compared against each other by calculating log ratios for each gene. Specifically, a lower signal threshold was set at 100 to reduce the amount of noise and another by calculating log ratios for each gene. Specifically, a lower signal threshold was set at 100 to reduce the amount of noise and another by calculating log ratios for each gene. Using Affymetrix MicroArray Suite 5.0 and an in-house Visual Basic software MATRIX 1.24 (Girard et al., manuscript in preparation). Briefly, array data were median normalised and samples (or averages of samples) were compared against each other by calculating log ratios for each gene. Specifically, a lower signal threshold was set at 100 to reduce the amount of noise and another by calculating log ratios for each gene. Using Affymetrix MicroArray Suite 5.0 and an in-house Visual Basic software MATRIX 1.24 (Girard et al., manuscript in preparation). Briefly, array data were median normalised and samples (or averages of samples) were compared against each other by calculating log ratios for each gene.
likely have been overlooked when using a CGH array with greater than 1 Mb resolution. In order to determine if the changes in array signal intensities reflect true copy number alterations, we selected three loci for FISH experiments. The loci and their associated clones’ locations are noted in Figure 3A. As seen in metaphase nuclei of NCI-H526, 548I13 shows three copies, whereas 476E5 shows two copies (Figure 3B). In a second experiment, 548I13 again shows three copies, whereas 385C11 shows six copies (Figure 3C). The normalised ratio of BAC 548I13 is at zero, even though it has three copies, as NCI-H526 has greater than a diploid

Table 1  SCLC cell lines analysed by array CGH and Affymetrix array (Phelps et al, 1996)

| Name       | Morphology | Sex | Age (years) | Source of specimen | Stage at diagnosis |
|------------|------------|-----|-------------|--------------------|-------------------|
| NCI-H82    | Variant    | M   | 41          | Pleural effusion   | Extensive         |
| NCI-H187   | Classical  | M   | 47          | Pleural effusion   | Extensive         |
| NCI-H289   | Variant    | F   | 45          | Pleural effusion   | Extensive         |
| NCI-H378   | Classical  | F   | 66          | Lung               | Extensive         |
| NCI-H526   | Variant    | M   | 55          | Bone marrow        | Extensive         |
| NCI-H889   | Classical  | F   | 69          | Lymph node         | Extensive         |
| NCI-H1184  | Classical  | M   | 42          | Lymph node         | Limited           |
| NCI-H1607  | Classical  | M   | 54          | Lymph node         | Extensive         |
| NCI-H1672  | Classical  | M   | 58          | Lung               | Limited           |
| NCI-H1963  | Classical  | M   | 56          | Lung               | Limited           |
| NCI-H2141  | Classical  | M   | 58          | Lymph node         | Extensive         |
| NCI-H2171  | Classical  | M   | 50          | Pleural effusion   | Extensive         |
| NCI-H2195  | Classical  | M   | 67          | Bone marrow        | Extensive         |
| NCI-H2227  | Classical  | M   | 54          | Lung               | Extensive         |
| HCC33      | Classical  | M   | 52          | NA                 | NA                |

SCLC = small-cell lung cancer; CGH = comparative genomic hybridisation; M = male; F = female; NA = not applicable.

Figure 1  Alignment of 1p array CGH profiles from 15 SCLC cell lines. Gains are shown to the right of the chromosome arm and losses to the left. Two recurrent amplifications are revealed, one at 1p34.2–p34.3 and the second at 1p11.2.
number of chromosomes. These data demonstrate the ability of array CGH to distinguish loci that differ in copy number by 0.67 times (3 vs 2) and two times (3 vs 6).

Two regions of sub-Mb amplification were common among 45–50% of the cell lines. Region 1 is a 580 kb amplification at 1p34.2–p34.3, and Region 2 is a 270 kb amplification at 1p11.2–p12. These two regions were detected in all but HCC33 were previously analysed for MYC amplification. The region contains 12 genes, listed in Table 2.

Conspicuously, MYCL1 amplification. The region contains 12 genes, listed in Table 2. Conspicuously, MYCL1, a gene first described in SCLC (Nau et al., 1985) is present in this region. Of the 15 cell lines described here, all but HCC33 were previously analysed for MYC family amplifications using Southern blot analysis (Johnson et al., 1996). NCI-H378 and NCI-H889 were described as having MYCL1 amplification, and in the current study, this amplification were also detected. We describe NCI-H526, NCI-H1184, NCI-H2141, and NCI-H1963 as having amplifications in the MYCL1 region; however, the Southern analysis did not find MYCL1 gene amplification in these cell lines. A possible explanation is that these cell lines do contain the amplification but they did not meet the four-fold signal increase cutoff necessary for the determination of an amplification in the Southern blot analysis. All other cell lines in which MYCL1 amplifications were not seen by Johnson et al. were also not seen amplified in the present study.

Previous conventional CGH studies of SCLC have revealed amplification in the MYCL1 region. In SCLC cell lines, Levin et al. (1994) detected amplification of chromosome bands 1p22–32 in nine of 18 samples (Levin et al., 1994). (MYCL1 was initially mapped to 1p32 (Nau et al., 1985), then reassigned to 1p34.3 (Speleman et al., 1996)). Ried et al. (1994) studied primary SCLC tumours and observed amplification of 1p32 in two of 13 cases (Ried et al., 1994). While these observations do implicate the MYCL1 locus, the regions identified are approximately 40 and 10 Mb in size, respectively, and contain hundreds of genes, making the identification of the specific genes affected by the amplification virtually impossible. Using high-resolution array CGH, we have defined the precise boundaries of the amplification in each cell line, with NCI-H2141 and HCC33 having amplified regions of less than 600 kb in size. We also determined that the minimal region of alteration at 1p34.2–p34.3 is 580 kb in size and contains 11 genes in addition to MYCL1. This demonstrates the resolving power of array CGH to identify very small alterations and to delineate minimal regions of alteration. Previous studies that focused only on the presence and not the extent of MYCL1 amplification may have overlooked the potential involvement of neighbouring genes.

The Region 2 amplification is a 270 kb amplification occurring in eight cell lines and contains BACs 385C11, 498H23, and 114O18. The 114O18 is the last BAC mapped to the centromeric end of the 1p arm. Three BACs used in FISH analysis are indicated. (B) FISH analysis of 548I13 vs 476E5, indicating that the presence of three copies of 548I13 and two copies of 476E5. (C) FISH analysis of 548I13 vs 385C11, indicating the presence of three copies of 548I13 and six copies of 385C11. FISH experiments were conducted as described previously (Henderson et al., 2004).
Table 2: Expression analysis of genes in Region 1 and Region 2 amplifications

| Gene       | Full name                                      | Affymetrix U133 probe set | Expression: amplification vs retention |
|------------|------------------------------------------------|---------------------------|----------------------------------------|
|            | Affymetrix ID | Accession | | SCLC vs normal | Affymetrix | RT–PCR | Overall score |
| Region 1   |              |           | | | | | |
| HEYL       | Hairy/enhancer- of-splitted related with       | 226828_s_at AL040198      | Yes | P = 0.00322 (up) | P = 0.47754 | P = 0.07599 | No change |
|            | YRPW motif like                                  |                           | No | — | — | — | |
| NTSC1/A    | 5’-nucleotidase, cystolic 1A                    | 220662_s_at NM_014571     | No | — | — | — | |
|            |                                                  | 224529_s_at AY028778      | No | — | — | — | |
| HPCAL4     | Hippocalin like 4                               | 222091_s_at AL136591      | Yes | P = 0.01098 (up) | P = 0.16783 | P = 0.34716 | No change |
| PPIE       | Peptidylprolyl isomerase E                      | 219671_at BE550384        | No | — | — | — | |
|            |                                                  | 229375_s_at AL526713      | Yes | P = 0.67684 (up) | P = 0.02704 | P = 0.23170 | No change |
|            |                                                  | 202494_at NM_001112       | Yes | P = 0.06631 (up) | P = 0.2317 | P = 0.0603 | No change |
|            |                                                  | 210502_s_at AF042386      | Yes | P = 0.84999 (up) | P = 0.2317 | P = 0.0603 | No change |
| BMP8       | Bone morphogenetic protein B                    | 207865_s_at NM_001720     | Yes | P = 0.00837 (up) | P = 0.01026 | P = 0.04693 (up) | Up |
|            |                                                  | 235275_at AA610122        | Yes | P = 0.00111 (up) | P = 0.00295 | | |
| OXCT2      | 3-oxoacid CoA transferase 2                     | 207866_s_at NM_001720     | No | — | — | — | |
| IPT        | TRNA isopentenyl transferase precursor          | 220256_s_at NM_022120     | No | — | — | — | |
|            |                                                  | 218617_at NM_017646       | Yes | P = 0.17806 | P = 0.00016 | P = 0.02005 (up) | Up |
| MYCL1      | Arm oncogene homolog 1, lung carcinoma derived  | Not included on array     | — | — | — | — | |
| FLJ14490   | Hypothetical protein FLJ14490                  | Not included on array | — | — | — | — | |
| CAP1       | Adenylyl cyclase-associated protein 1           | 200625_s_at NM_006367     | Yes | P = 0.00623 (down) | P = 0.43326 | P = 0.38943 | No change |
|            |                                                  | 213798_s_at AA806142      | Yes | P = 0.00623 (down) | P = 0.14048 | | No change |
| PPT1       | Palmitoyl–protein thioesterase 1               | 200975_s_at NM_000310     | Yes | P = 0.15369 (up) | P = 0.2317 | | No change |
| RLF        | Rearranged L-myc fusion sequence               | 204243_s_at NM_012421     | Yes | P = 0.79098 (up) | P = 0.00699 | P = 0.03605 (up) | Up |
| Region 2   |              |           | | | | | |
| ADAM30     | A disintegrin and metalloproteinase domain 30   | 221446_s_at NM_021794     | No | — | — | — | |
| Notch2     | Notch homolog 2                                | 202445_s_at NM_024408     | No | — | — | — | |
|            |                                                  | 202443_s_at AA291203      | Yes | P = 0.00004 (down) | P = 0.16783 | P = 0.06030 | No change |
|            |                                                  | 212377_s_at AU158495      | Yes | P = 0.00015 (down) | P = 0.26791 | | No change |

SCLC = small cell lung cancer; RT–PCR = reverse transcriptase–polymerase chain reaction. *Affymetrix score of Present or Marginal in > 50% of samples. **RT–PCR analysis failed to confirm the expression difference detected by Affymetrix.

1p arm. Two genes, ADAM30 and Notch2, reside in this region. ADAM30 is a member of the family of membrane proteins that contain a disintegrin and metalloprotease domain and its normal expression has only been detected in the testis (Cerretti et al., 1999). While ADAM30 itself has not been implicated in cancer, a number of other ADAM genes have, such as ADAM9 in breast cancer (O’Shea et al., 2003) and ADAM12 in bone cancer (Tian et al., 2002). Notch2 and the three other genes in its family, Notch1, Notch3, and Notch4, code for evolutionarily conserved Type 1 transmembrane receptor proteins (Artavanis-Tsakonas et al., 1999). While these proteins have been widely studied, investigations into their possible roles in cancer have yielded a number of studies with differing conclusions. Notch1 was first described as being involved in a balanced translocation with TCRβ and a constitutively active Notch1 was oncogenic in T cells (Ellisen et al., 1991). Notch3 overexpression has been shown in NSCLC (Dang et al., 2000); however, it has also been demonstrated that expression of Notch1 and Notch2 induced cell cycle arrest in SCLC cell lines (Sriuranpong et al., 2001).

We searched for alterations of Regions 1 and 2 in NSCLC. In the analysis of nine NSCLC cell lines, we detected amplification of Region 1 in one sample and Region 2 in four samples (data not shown). In comparison to SCLC, the amplifications in NSCLC included larger regions and, in the case of Region 1, were at a lower level. For example, NCI-H520, a lung squamous cell carcinoma, has a 7 Mb amplification containing Region 1, and a 3.2 Mb amplification containing Region 2. The lower frequency of Region 1 amplification is in agreement with a previous observation that MYC family gene amplification occurs at a frequency of less than 5% in NSCLC (Bruce Johnson, personal communication), as opposed to up to 36% in SCLC (Johnson et al., 1996). The amplification of Region 2 was also detected in NSCLC tumours, where overexpression of Notch2 was observed (Garnis et al., 2005).
Gene expression analysis for each of these cell lines was obtained using Affymetrix GeneChips HG-U133A and HG-U133B. Expression levels of the genes contained in the Region 1 and Region 2 amplifications were compared between the SCLC cell lines and normal control cell lines SAEC, NHBECl, and four immortalised HBEC lines. Additionally, as there is no normal tissue that is known to be a suitable control for gene expression in SCLC, we also compared gene expression among cell lines with and without amplifications in the regions of interest. It has been previously demonstrated that the overexpression of some of the genes in a tumour can be attributed to their genomic amplification. Up to 44% of amplified genes (depending on their level of amplification) were found to be overexpressed in breast cancer cell lines (Hyman et al., 2002). We further compared the expression levels of these two sets of SCLC cell lines using semiquantitative RT–PCR analysis for the genes where a significant difference in expression was observed in the Affymetrix data. The results for all analyses of gene expression are detailed in Table 2 and a comparison of relative expression levels is displayed in Figure 4.

In Region 2, ADAM30 expression was not detected on the Affymetrix array. Notch2 was underexpressed in SCLC compared to normal cell lines. This observation is consistent with the observations of Suriarapong et al. (2001), where overexpression of Notch2 in SCLC induced cell cycle arrest. No difference in Notch2 expression was observed between SCLC cells with and without the genomic amplification of Region 2 in either Affymetrix or RT–PCR analyses (see Figure 4B and Table 2). It appears that the amplification of this region may not have a functional role in increasing the expression of the genes contained in it.

A number of expression differences in both comparisons were observed for the genes in Region 1. In comparing SCLC and normal cells, four genes demonstrated significant differences: HEYL, HPCAL4, BMP8, and CAP1. CAP1 showed underexpression, while the other three were overexpressed. The comparison of SCLC cells with and without amplification of Region 1 revealed three genes that were overexpressed in the amplified cells: BMP8, IPT, and RLF. One gene, PPIE, was observed overexpressed in one of its three Affymetrix probes, but no difference was observed in its other two probes or the RT–PCR analysis. This may indicate a problem with that particular probe on the Affymetrix array. All other genes for which RT–PCR analysis was conducted agreed with the Affymetrix analysis (see Table 2). BMP8 was the only gene overexpressed in both comparisons. Owing to the possibility of expression between SCLC and normal cells being skewed by extremely high expression in the amplified samples, we compared BMP8 expression between the normal cell lines and the nonamplified SCLC cells. BMP8 remained overexpressed in SCLC (data not shown).

HEYL is part of a subfamily of bHLH (basic helix–loop–helix) transcription factors. These proteins control cell fate decisions such as segmentation, neurogenesis, and myogenesis (Steidl et al., 2000). The mouse homologue of HEYL has been shown to be a target of Notch1 signalling during development (Leimeister et al., 2000). As mentioned above, the Notch pathway has been found to have differing roles in cancer, depending on the tumour type. HPCAL4 expression has only been detected in the brain. It is part of neuron-specific calcium-binding protein family; however, the specific function of HPCAL4 is not known (Kobayashi et al., 1998). HPCAL4 expression was not present in the normal lung cell lines (based on the detection P-value), but expression was seen in the SCLC cells. This may be a result of the neuroendocrine nature of SCLC.

CAP1 is associated with actin and coflin and allows the rapid turnover of actin filaments. This is an important function in cell motility. In yeast, CAP is a component of the Ras pathway; however, this role has not been identified in humans (Moriyama and Yahara, 2002). Differential expression of CAP1 has not been previously detected in cancer; however, a potential role for the actin and coflin complexes may be increasing cell motility during metastasis. A role such as this would imply overexpression of the gene involved, whereas we have observed underexpression of CAP1.

BMP8 is a bone morphogenetic protein, part of the TGF-β superfamily. These proteins play a role in aspects of mammalian development such as mesoderm determination, neural patterning, organogenesis, and skeletal patterning (DiLeone et al., 1997). There have been a number of studies of BMPs in cancer. BMPs were identified as potential tumour suppressor genes in myeloma (Hjertner et al., 2001) and prostate cancer (Brubaker et al., 2004). They were also shown to be overexpressed in oral squamous cell carcinoma (Jin et al., 2001) and BMP7 was overexpressed in breast cancer cell lines (Hyman et al., 2002).

RLF has been shown to be in fusion with MYC1 in SCLC. Kim et al. (1998) detected genomic amplification of RLF in four of 11 MYC1-amplified SCLC cell lines they studied. Chimeric RLF-MYC1 transcripts were detected in NCI-H889, NCI-H1836, and NCI-H1994 (Kim et al., 1998). RLF contains zinc-finger motifs, and is related to the Zn-15 transcription factor. It is thought to have a

![Figure 4](image-url)
general role in transcriptional regulation (Makela et al, 1995). The RLF-MYCL1 fusion protein has also been detected in SCLC primary tumours. Its role is thought to be the deregulation of expression of MYCL1 (Makela et al, 1992). Interestingly, while RLF was originally identified due to fusion and overexpression with MYCL1, it was not the cell lines with the highest amplification in which we observed the highest expression. While NCI-H378 and NCI-H889 show the highest amplification of the region, and were the only two cell lines with previously known MYCL1 amplification, NCI-H1184 and NCI-H1963 both express RLF at higher levels than NCI-H378 and NCI-H889. The observation of increased expression of RLF, particularly in cell lines that had not been previously studied, lends additional weight to its implication in SCLC.

The overexpression of IPT was the most significantly associated with amplification (see Figure 4 and Table 2). IPT catalyses the transfer of an isopentenyl group from dimethylallyl pyrophosphate to a tRNA in the biosynthesis of the tRNA cytokinin isopentenyladenosine (Golovko et al, 2000). Isopentenyladenine is an end product of the mevalonate pathway. Mevalonate is the precursor of isoprenoid groups that are incorporated into other end products in addition to isopentenyladenine such as sterols and ubiquinone (Goldstein and Brown, 1990). HMG-CoA reductase catalyses the conversion of HMG-CoA to mevalonate. Elevated activity of this protein has been observed in a number of tumour types. The statin family of drugs are HMG-CoA reductase inhibitors that reduce levels of mevalonate and its end products. Statins have been successfully used in the treatment of hypercholesterolaemia, and have been recently demonstrated to have antiproliferative and proapoptotic effects in tumours both in vitro and in vivo (Wong et al, 2002). As the mevalonate pathway has been shown to play an important role in the maintenance of the malignant phenotype, IPT, which contributes to the production of an end product, may have a role in promoting malignancy as well. Its specific function and contribution remain to be clarified.

We have presented a chromosome arm-specific tiling resolution BAC array consisting of 642 BAC clones spanning 120 Mb of chromosome arm 1p and profiled 15 SCLC cell lines using this array. We have reliably detected the previously known MYCL1 amplification, as well as defined a 580 kb amplification at 1p34.2–p34.3, and a novel 270 Kb Amplification at 1p11.2. This demonstrates the ability of high-resolution array CGH to identify small alterations that may have escaped detection by other means, and to detect efficiently the chromosomal location, size, and relative level of a genetic alteration. Further, we have analysed the expression of the genes contained in the amplicons, in order to identify those with a potential role in SCLC. Notch2 and CAP1 were underexpressed, while HEYL, HPCAL4, and BMP8 were overexpressed in SCLC in comparison to normal cell lines. IPT, BMP8, and RLF were overexpressed in SCLC cells in which they were genomically amplified in comparison to SCLC cells without amplification. These data are in agreement with previous studies that broadly implicate the BMP family in cancer, and specifically implicate RLF in SCLC. Furthermore, we have observed increased expression of HEYL, HPCAL4, and IPT, for which additional investigation will be necessary to define their roles in cancer.

Genomic amplification is significant to cancer in that it can directly result in increased expression of amplified genes (Hyman et al, 2002). High-level amplifications have usually been seen associated with a particular oncogene such as EGRF, MYC, or ERBB2 (Schwab, 1999). Here, we have described an amplicon containing the MYCL1 oncogene, where additional genes are affected by the genomic amplification. This demonstrates the importance of integrating comprehensive genomic and gene expression analyses where all genes in a region can be studied. Further, we have demonstrated the benefit of a comparison among samples of the same tumour type as well as against normal tissues in order to reveal expression changes that may be functionally important.

ACKNOWLEDGEMENTS

We wish to acknowledge the contributions of Tiffany Wolters, JinHee Kim, Emily Vucic, Timon Buys, Chad Malloff, Spencer Watson, and Adrian Ishkanian. This work was supported by grants from Genome Canada, Genome British Columbia, the National Cancer Institute of Canada, the Canadian Institute for Health Research, and Lung SPORE P50CA70907. BPC is supported by a scholarship from the Michael Smith Foundation for Health Research.

REFERENCES

Artavanis-Tsakonas S, Rand MD, Lake RJ (1999) Notch signaling: cell fate control and signal integration in development. Science 284: 770 – 776
Ashman JN, Brigham J, Cowen ME, Bahia H, Greenman J, Lind M, Cawkwell L (2002) Chromosomal alterations in small cell lung cancer revealed by multicolour fluorescence in situ hybridization. Int J Cancer 102: 230 – 236
Brubaker KD, Corey E, Brown LG, Vessella RL (2004) Bone morphogenetic protein signaling in prostate cancer cell lines. J Cell Biochem 91: 151 – 160
Buckley PG, Mantripragada KK, Benetkiewicz M, Tapia-Paez I, Diaz De Ashman JN, Brigham J, Cowen ME, Bahia H, Greenman J, Lind M, Cawkwell L (2002) Chromosomal alterations in small cell lung cancer revealed by multicolour fluorescence in situ hybridization. Int J Cancer 102: 230 – 236
Ellison LW, Bird J, West DG, Soreng AL, Reynolds TC, Smith SD, Sklar J (1991) TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic leukaemias. Cell 66: 649 – 661
Garnis C, Baldwin C, Zhang L, Rosin MP, Lam WL (2003) Use of complete coverage array comparative genomic hybridization to define copy number alterations on chromosome 3p in oral squamous cell carcinomas. Cancer Res 63: 8852 – 8858
Garnis C, Campbell J, Davies JJ, Macauley C, Lam S, Lam WL (2005) Involvement of multiple developmental genes on chromosome 1p in lung tumorigenesis. Hum Mol Genet 14: 475 – 482
Garnis C, Coe BP, Ishkanian A, Zhang L, Rosin MP, Lam WL (2004a) Novel regions of amplification on 8q distinct from the MYC locus and frequently altered in oral dysplasia and cancer. Genes Chromosomes Cancer 39: 93 – 98
Garnis C, Coe BP, Zhang L, Rosin MP, Lam WL (2004b) Overexpression of LRPL2, a gene contained within an 8q22 amplicon identified by high-resolution array CGH analysis of oral squamous cell carcinomas. Oncogene 23: 2582 – 2586
Gazdar AF, Kurvuri V, Virmani A, Gollahon L, Sakaguchi M, Westerfield M, Kodagoda D, Stanny V, Cunningham HT, Wistuba II, Tomlinson G, Tonn V, Ashfaq R, Leitch AM, Minna JD, Shay JW (1998) Characterization of paired tumor and non-tumor cell lines established from patients with breast cancer. *Int J Cancer* 78: 766 – 774

Girard L, Zochbauer-Muller S, Virmani AK, Gazdar AF, Minna JD (2000) Genome-wide allelotyping of lung cancer identifies new regions of allelic loss, differences between small cell lung cancer and non-small cell lung cancer, and loci clustering. *Cancer Res* 60: 4894 – 4906

Goldstein JL, Brown MS (1990) Regulation of the mevalonate pathway. *Nature* 343: 425 – 430

Golovko A, Hjalm G, Sitbon F, Nicander B (2000) Cloning of a human RNA isopentenyl transferase. *Gene* 258: 85 – 93

Greshock J, Naylor TL, Margolin A, Diskin S, Cleaver SH, Futreal PA, Dejong PJ, Zhao S, Liebman M, Weber BL (2004) I-Mb resolution array-based comparative genomic hybridization using a BAC clone set optimized for cancer gene analysis. *Genome Res* 14: 179 – 187

Henderson L, Okamoto I, Lestou VS, Lubkovski O, Robichaud M, Chhanabhai M, Gascoyne RD, Klasa RJ, Connors JM, Marra MA, Horsman DE, Lam WL (2004) Delineation of a minimal region of deletion at 6q16.3 in follicular lymphoma and construction of a bacterial artificial chromosome contig spanning a 6-megabase region of 6q16–q21. *Genes Chromosomes Cancer* 40: 60 – 65

Hjertner O, Hjorth-Hansen H, Borset M, Seidel C, Waage A, Sundan A (2001) Bone morphogenetic protein-4 inhibits proliferation and induces apoptosis of multiple myeloma cells. *Blood* 97: 516 – 522

Hyman E, Kauraniemi P, Hautaniemi S, Wolf M, Mousses S, Rozenbult E, Ringner M, Sauter G, Monni O, Elkahloun A, Kallioniemi OP, Kallioniemi A (2002) Impact of DNA amplification on gene expression patterns in breast cancer. *Cancer Res* 62: 6240 – 6245

International Human Genome Mapping Consortium (2001) A physical map of the human genome. *Nature* 409: 934 – 941

Ishkanian AS, Malloff CA, Watson SK, DeLeouw RJ, Chi B, Coe BP, Snijders AM, Albertson DG, Pinkel D, Marra MA, Ling V, Maculay C, Lam WL (2004) A tiling resolution DNA microarray with complete coverage of the human genome. *Nat Genet* 36: 299 – 303

Jin Y, Tipoe GL, Liong EC, Lau TY, Fung PC, Leung KM (2001) Overexpression of BMP-2/4, -5 and BMPR-IA associated with malignancy of oral epithelium. *Oral Oncol* 37: 225 – 233

Johnson BE, Russell E, Simmons AM, Phelps R, Steinberg SM, Iddc DL, Gazdar AF (1996) MYC family DNA amplification in 126 tumor cell lines optimized for cancer gene analysis. *Genome Res* 9: 1718 – 1726

Kent WJ, Sugnet CW, Furey TS, Roskin TH, Zabih AM, Haussler D (2002) The human genome browser at UCSC. *Genome Res* 12: 996 – 1006

Kim JO, Nau MM, Allikian KA, Makela TP, Altaito K, Johnson BE, Kelley MJ (1998) Co-amplification of a novel cyclolin-like gene (PPIE) with L-myc in small cell lung cancer cell lines. *Oncogene* 17: 1019 – 1026

Kobayashi M, Saki E, Furuta Y, Takamatsu K (1998) Isolation of two calcium-binding protein genes. *DNA Seq* 9: 171 – 176

Leimister C, Schumacher N, Steidl C, Gessler M (2000) Analysis of HEY1 expression in wild-type and Notch pathway mutant mouse embryos. *Mech Dev* 98: 175 – 178

Levin NA, Brzoska P, Gupta N, Minna JD, Gray JW, Christman MF (1994) Identification of frequent novel genetic alterations in small cell lung cancer. *Cancer Res* 54: 5086 – 5091

Makela TP, Hellsten E, Vesa J, Hirvonen H, Palotie A, Petlonen L, Altaito K (1995) The rearranged L-myc fusion gene (RLF) encodes a Zn-15 related zinc finger protein. *Oncogene* 11: 2699 – 2704

Makela TP, Shiraiishi M, Borrello MG, Sekiya T, Altaito K (1992) Rearrangement and co-amplification of L-myc and rlf in primary lung cancer. *Oncogene* 7: 405 – 409

Marr MA, Kucaba TA, Dietrich NL, Green ED, Brownstein B, Wilson RK, McDonald KM, Hillier LW, McPherson JD, Waterston RH (1997) High throughput fingerprint analysis of large-insert clones. *Genome Res* 7: 1072 – 1084

Minna JD, Kurie JM, Jacks T (2003) A big step in the study of small cell lung cancer. *Cancer Cell* 4: 163 – 166

Moriyama K, Yahara I (2002) Human CAP1 is a key factor in the recycling of cofilin and actin for rapid actin turnover. *J Cell Sci* 115: 1591 – 1601

Nau MM, Brooks BJ, Battey J, Sausville E, Gazdar AF, Kirsh IR, McBride OW, Birtess V, Hollis GF, Minna JD (1985) L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer. *Nature* 318: 69 – 73

Nomoto S, Haruki N, Tatematsu Y, Konishi H, Mitsudomi T, Takahashi T, Hori K, Ito S, Nakamura A, Iwai A, Minna JD (2000) Frequent allelic imbalance suggests involvement of a tumor suppressor gene at 1p36 in the pathogenesis of human lung cancers. *Genes Chromosomes Cancer* 28: 342 – 346

O’Shea C, McKie N, Buggy G, Duggan C, Hill AD, McDermott E, O’Higgins N, Duffy MJ (2003) Expression of ADAM-9 mRNA and protein in human breast cancer. *Int J Cancer* 105: 754 – 761

Oseogawa K, Mammoser AG, Wu C, Frengen E, Zeng C, Catanese JJ, de Jong PJ (2001) A bacterial artificial chromosome library for sequencing the complete human genome. *Genome Res* 11: 483 – 496

Phillips RM, Johnson BE, Iddc DL, Gazdar AF, Carbone DP, McClintock PR, Linnola RJ, Matthews MJ, Bunn Jr PA, Carney D, Minna JD, Mushline JL (1996) NCI-Navy Medical Oncology Branch cell line database. *J Cell Biochem Suppl* 24: 32 – 91

Pinkel D, Segraves R, Sudar D, Clark S, Poole I, Kowbel D, Collins K, Kuo WL, Chen C, Zhay D, Daikree SH, Lijung BM, Gray JW, Albertson DG (1998) High resolution analysis of DNA copy number variation using comparative genomic hybridization to microarrays. *Nat Genet* 20: 207 – 211

Ramirez RD, Sheridan S, Girard L, Sato M, Kim Y, Pollack J, Peyton M, Zou Y, Kurie JM, Dimajo JM, Milchgrub S, Smith AL, Souza RF, Gilby L, Zhang X, Gandia K, Vaughan MB, Wright WE, Gazdar AF, Shaw JY, Minna JD (2004) Immortalization of human bronchial epithelial cells in the absence of viral oncoproteins. *Cancer Res* 64: 9027 – 9034

Ried T, Petersen I, Holtgreve-Greuz H, Speicher MR, Schrock E, du Manoir S, Cremer T (1994) Mapping of multiple DNA gains and losses in primary small cell lung carcinomas by comparative genomic hybridization. *Cancer Res* 54: 1801 – 1806

Schwab M (1999) Oncogene amplification in solid tumors. *Semin Cancer Biol* 9: 319 – 325

Snijders AM, Nowak N, Segraves R, Blackwood S, Brown N, Conroy J, Hamilton G, Hiddle AK, Huey B, Kimura K, Law S, Myamoto K, Palmer J, Ylstra B, Yue JP, Gray JW, Jain AN, Pinkel D, Albertson DG (2001) Assembly of microarrays for genome-wide measurement of DNA copy number. *Nat Genet* 29: 263 – 264

Speleman F, Van Camp G, Van Roy N (1996) Reassignment of MYCL1 to human chromosome 1p34.3 by fluorescence in situ hybridization. *CytoGenet Cell Genet* 72: 189 – 190

Sriuranpong V, Borges MW, Ravi RK, Arnold DR, Nelkin BD, Baylin SB, Ball DW (2001) Notch signaling induces cell cycle arrest in small cell lung cancer cells. *Cancer Res* 61: 3200 – 3205

Steidl C, Leimister C, Klamt B, Maier M, Nanda I, Dixon M, Clarke R, Schmid M, Gessler M (2000) Characterization of the human and mouse HEY1, HEY2, and HEY1 genes: cloning, mapping, and mutation screening of a new BHH gene family. *Genomics* 66: 195 – 203

Tsai BL, Wen JM, Zhang M, Xie D, Xu RB, Luo CJ (2002) The expression of ADAM12 (meltrin alpha) in human giant cell tumours of bone. *Mol Pathol* 55: 394 – 397

Wong WW, Dimitroulakis J, Minden MD, Penn LZ (2002) HMG-CoA reductase inhibitors and the malignant cell: the statin family of drugs as triggers of tumor-specific apoptosis. *Leukemia* 16: 508 – 519