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Small-cell lung cancer is characterized by a high incidence of deletions on chromosomes 3p, 4q, 5q, 10q, 13q and 17p

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Summary The genetic mechanisms that define the malignant behaviour of small-cell lung cancer (SCLC) are poorly understood. We performed comparative genomic hybridization (CGH) on 22 autoptic SCLCs to screen the tumour genome for genomic imbalances. DNA loss of chromosome 3p was a basic alteration that occurred in all tumours. Additionally, deletions were observed on chromosome 10q in 94% of tumours and on chromosomes 4q, 5q, 13q and 17p in 86% of tumours. DNA loss was confirmed by loss of heterozygosity (LOH) analysis for chromosomes 3p, 5q and 10q. Simultaneous mutations of these six most abundant genetic changes were found in 12 cases. One single tumour carried at least five deletions. DNA under-representations were observed less frequently on chromosome 15q (55%) and chromosome 16q (45%). The prevalent imbalances were clearly indicated by the superposition of the 22 tumours to a CGH superkaryogram. In our view, the high incidence of chromosomal loss is an indication that SCLC is defined by a pattern of deletions and that the inactivation of multiple growth-inhibitory pathways contributes in particular to the aggressive phenotype of that type of tumour.

Keywords: small-cell lung cancer; lung cancer; comparative genomic hybridization; loss of heterozygosity; tumour genetics

Small-cell lung cancer (SCLC) accounts for about 18% of all primary lung carcinomas (Ries et al, 1991), and the incidence is rising especially in women (El-Torky et al, 1990). The mean survival after diagnosis ranges between 6 and 18 months (Fox and Scadding, 1973; Johnson et al, 1978; Medical Research Council, 1979), and SCLC is thus one of the most malignant tumours in man. It may present itself as an acute disease with respiratory insufficiency owing to a tumour bulk in the central bronchi associated with post-obstructive pneumonitis. The interval between symptoms and diagnosis is shorter than in other types of lung cancer. As dissemination occurs early, SCLC is usually considered a systemic disease. This has lead to a therapeutic regimen that avoids any surgical treatment and is based solely on chemotherapy and radiotherapy.

Cytogenetic studies indicated almost 14 years ago that a deletion on chromosome 3p is a characteristic finding in SCLC (Whang-Peng et al, 1982). Although this was confirmed by molecular genetic and molecular cytogenetic studies (Yokota et al, 1987; Mori et al, 1989; Levin et al, 1994; Ried et al, 1994), 3p loss alone is not sufficient to define SCLC, since it is also a frequent change in other tumour entities, e.g. non-small-cell lung cancer (Tsuchiya et al, 1992; Sato et al 1994), head and neck squamous cell carcinoma (Broszka et al, 1995; Speicher et al, 1995), bladder cancer (Rodriguez et al, 1994) and cervix carcinomas (Yokota et al, 1989). In addition, deletions on chromosomes 5q, 13q and 17p have previously been reported in SCLC (Yokota et al, 1987; Mori et al, 1989; Miura et al, 1992). Candidate tumour-suppressor genes (TSGs) that are frequently inactivated are the retinoblastoma gene *Rb1* at 13q14 (Kelley et al, 1995) and *p53* at 17p13 (Sameshima et al, 1992).

The amplification of *myc* oncogenes has been associated with advanced tumour stages in SCLC (Takahashi et al, 1989) and the overexpression of the *c-myc* oncoprotein correlated with poor prognosis in head and neck squamous cell carcinomas (Field et al, 1989). It is questionable, however, whether the evalution of single genetic events is sufficient to define the malignant potential of a tumour.

In the present study, we used comparative genomic hybridization (CGH) to screen the genomes of 22 autoptic SCLCs for genetic alterations. The analysis suggests that SCLC is characterized by a pattern of alterations that include especially deletions on chromosomes 3p, 4q, 5q, 10q, 13q and 17p. DNA loss of chromosome 10q is associated with tumour progression in gliomas (Leenstra et al, 1994), menigeomas (Rempel et al, 1993) and endometrial carcinomas (Peiffer et al, 1995). Accordingly, it might contribute in particular to the malignant phenotype of SCLC.

MATERIALS AND METHODS

Tumour specimens

The tumours were collected at autopsy perfomed between 8 and 36 h after the patients' death. The specimens were frozen in liquid nitrogen and stored at –80°C until DNA was extracted. The clinicopathological data are shown in Table 1. Fourteen patients were women and eight were men. The majority showed advanced tumour stages. The primary tumour was investigated in 18 cases. In four cases, the tumour specimens were derived from metastatic lesions, i.e. in case 13 from a paratracheal lymph node metastasis, in cases 3 and 15 from a liver metastasis and in case 4 from a pleural metastasis. Thirteen tumours that had been previously analysed

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Table 1 Clinicopathological data

Case	Sex	Age (Years)	Stage
1	F	63	pT3 pN3 pM1
2	F	61	pT2 pN1 pM0
3	M	71	pT3 pN2 pM1
4	F	59	pT4 pN3 pM1
5	F	46	pT4 pN3 pM1
6	M	83	pT3 pN3 pM1
7	F	57	pT3 pN2 pM1
8	M	50	pT4 pN1 pM1
9	M	75	pT4 pN3 pM1
10	F	80	pT2 pN2 pM1
11	F	66	pT3 pN3 pM1
12	F	67	pT2 pN0 pM0
13	F	56	pT4 pN3 pM1
14	F	58	pT4 pN3 pM1
15	M	55	pT3 pN1 pM1
16	M	80	pT4 pN3 pM1
17	F	57	pT3 pN3 pM1
18	M	78	pT3 pN3 pM1
19	F	33	pT3 pN2 pM1
20	F	79	pT4 pN3 pM1
21	F	70	pT4 pN3 pM0
22	M	75	pT2 pN3 pM0

(Ried et al, 1994) were rehybridized and assessed by a different CGH analysis software (Wolf et al, 1995; Roth et al, 1996).

CGH preparation

Normal metaphase chromosomes were prepared from peripheral blood lymphocytes by standard procedures. In general, no pepsin or protease treatment was applied. The metaphase chromosomes were denatured for 90 s in 70% formamide/2 × saline sodium citrate (SSC) at 77°C and dehydrated by an increasing ethanol series (70%, 90%, 100% EtOH). Each batch of metaphases was tested by hybridization with differentially labelled normal DNA as described (Kallioniemi et al, 1994). The tumour and normal DNA were labelled by nick translation with biotin-dUTP and digoxigenin-dUTP (Böhringer Mannheim, Mannheim, Germany) respectively. Tumour (1μg) and 1μg of normal DNA plus 20 μg of human Cot1 DNA (Gibco BRL, Life Technologies, Paisley, UK) was ethanol precipitated and the DNA pellet was resuspended in 5 μl of formamide. After adding 10 μl of master mix (20% dextran sulphate/4 × SSC), the DNA was denatured for 5 min at 77°C and prehybridized at 37°C for 1 h. Finally, 12 μl was applied to the slide with the denatured and dehydrated metaphase chromosomes. Hybridization was done under a 18 × 18 mm coverslip that was sealed with rubber cement for 3 days at 37°C. The detection of the genomes was performed by fluorescein–avidin (Vector Laboratories, Burlington, CA, USA) and anti-digoxigenin–rhodamine (Böhringer Mannheim) without fluorescence enhancement (Ried et al, 1992). DAPI was used for chromosome counterstaining.

CGH image acquisition and digital image analysis

Three images per metaphase (DAPI, fluorescein isothiocyanate (FITC), tetrarhodamine isothiocyanate (TRITC)) were acquired on an Axiophot epifluorescence microscope (Zeiss, Oberkochen, Germany) by using appropriate filter sets (DAPI: Zeiss filter set 02, i.e. excitation G365, beam splitter FT395, emission LP 420; FITC: Zeiss filter set 20, i.e. excitation BP 450–490, beam splitter FT 510, emission BP 546/12; TRITC: Chroma filter set HQ Cy3 plus excitation filter of Zeiss filter set 15, i.e. excitation BP 546/12, beam splitter FT 565, emission BP 570–650). The images were stored under the TIFF format and analysed by a custom-made CGH analysis program (Wolf et al, 1995; Roth et al, 1996). It involves a karyotyping program (KARYOTYP, IBSB, Berlin) that is based on the digital image analysis software AMBA, which was developed in our laboratory (Roth et al, 1992). The program is compatible with Windows 3.1 and Windows 95 (Microsoft, Redmont, WA, USA). Briefly, the digital image analysis comprised the following steps:

1. definition of the image objects (chromosomes) by segmentation of the inverted DAPI image;
2. loading of the FITC and TRITC image under the DAPI segmentation mask;
3. correction of the optical shift of the FITC and TRITC image;
4. calculation of the RATIO (FITC/TRITC) image;
5. separation of touching chromosomes;
6. karyotyping of the DAPI chromosomes (the FITC, TRITC and RATIO chromosomes can be displayed during the karyotyping process by means of a 'compare' function); and
7. calculation of the mean ratio chromosomes (CGH sum-karyogram) and the mean ratio profiles by averaging at least five metaphases/karyograms.

In the CGH sum-karyogram (Figure 1A), a particular mean ratio chromosome is calculated from all chromosomes of the same class that are present in the individual karyograms (Roth et al, 1996). The CGH superkaryogram (Figure 3) is an extension of this concept. A chromosome in CGH superkaryogram is calculated by averaging the chromosomes of the same class of CGH sum-karyograms of different tumours that belong to a specific tumour entity (Manuscript in preparation).

LOH analysis

Paired samples of tumour and normal DNA were assessed for allelic loss by microsatellite polymorphism or polymerase chain reaction – restriction fragment length polymorphism (PCR–RFLP) analysis (Petersen et al, 1993). We investigated up to five different markers on chromosome 3p that were located at 3p13 (D3S659), 3p13–14 (D3S30), 3p23 (D3S647) and 3p24 (THRB, EA2B), three markers on chromosome 5q near the APC locus at 5q21–22 and two markers on chromosome 10q (D10S169 and D10S1213). D10S1213 is 18 cM centromic of D10S169, which was mapped to the chromosomal band 10q26. The primer sequences and the PCR conditions were used as published (Cottrell and Bodmer, 1992; Ganly and Rabbitts, 1992*a,b*; Koorey et al, 1992; Miyoshi et al, 1992; Sakurai et al, 1992) or extracted from the Genome Database via Internet (URL: http://gdbwww.gdb.org/). We used a non-radioactive detection for the assessment of microsatellite polymorphisms (Petersen et al, 1996).

RESULTS

The result of the CGH analysis of case 3 is shown in Figure 1. The CGH sum-karyogram (Figure 1A) and the ratio profile with the 95% confidence interval (Figure 1B) were primarily used to define the genetic changes in a particular tumour. If the mean ratio profile was to the left of the monosomy threshold, it was considered a deletion. If the mean ratio profile was to the right of the trisomy

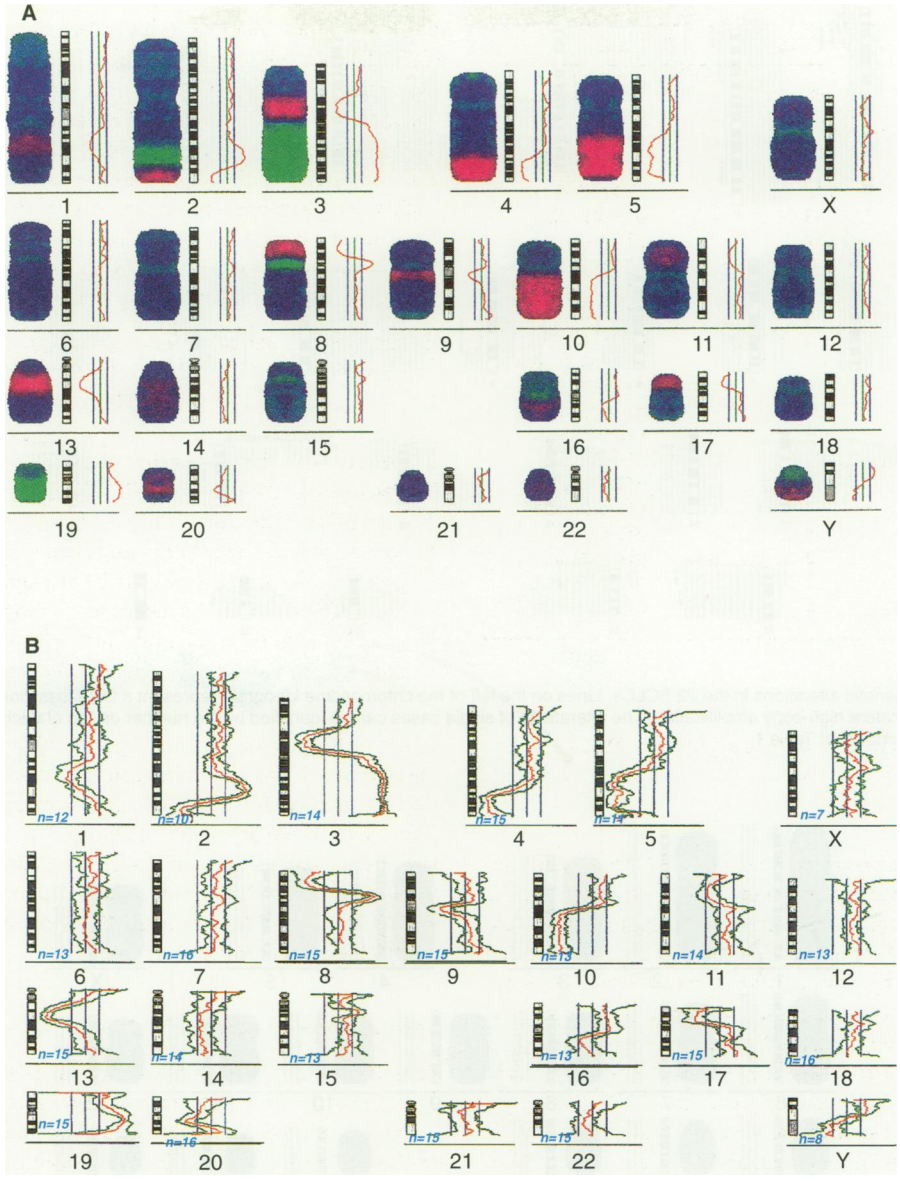

Figure 1 (**A**) CGH sum-karyogram of case 3. The chromosomes of eight metaphases/karyograms were included in the analysis. Deletions are depicted in red, amplifications in green and equilibrium between the tumour and normal DNA in blue. Changes include deletions on chromosomes 3p11–14, 4q31–qter, 5q21–35, 10q21–qter, 13q11–14 and 17p11–pter. In addition, amplifications are present on chromosomes 2q32–34, 3q11–qter, 8p11 and on chromosome 19. (**B**) Ratio profile of case 3 with the 95% confidence interval. The ratio profile represents the result as a one-dimensional curve. The three lines to the right of the chromosome ideogram represent different fluorescence ratios, i.e. 0.75, 1 and 1.25. The central line corresponds to the normal state (fluorescence ratio 1:1). The lines to the left and right represent the theoretical values of a monosomy or trisomy in 50% of the tumour cells of an otherwise diploid tumour. The number of chromosomes analysed are indicated at each chromosome ideogram

threshold, it was judged as an over-representation. An over-representation was defined as a high-copy amplification, if the fluorescence ratio exceeded at least the value of 1.5. Every alteration was re-evaluated by visual inspection of all chromosomes of the karyograms that were included in the CGH sum-karyogram. In general, the 13 tumours that had been investigated previously by a different CGH image analysis software (Ried et al, 1994) revealed similar results. In one case (no. 13), however, we observed a global profile shift, i.e. the ratio profiles of all chromosomes were displaced toward DNA loss by a fluorescence ratio value of about 0.5. Thus, those chromosomes that suggested no DNA loss in the first analysis because the profiles were on the central line (fluorescence ratio 1:1) now indicated a DNA under-representation (fluorescence ratio 0.5), whereas the other chromosomes, which showed an over-representation, were normal in the present analysis. The reason for the profile shift was owing to a misdefinition of the so-called central line by the previously used program (Thomas Ried, personal communication).

The summary of all alterations with reference to individual cases is shown in Figure 2. In Figure 3, the aberrations of all cases were compressed to a CGH superkaryogram representing typical changes of the tumour entity.

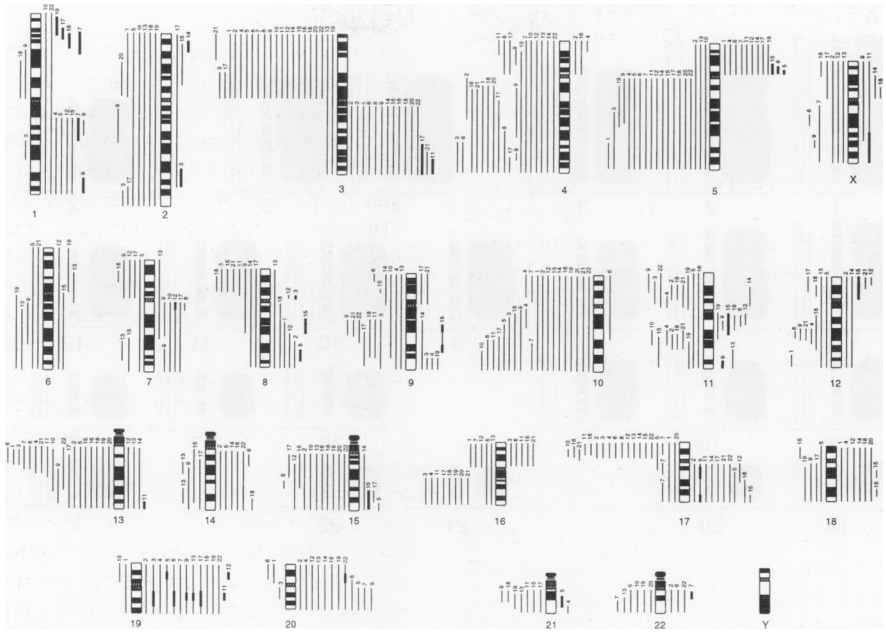

Figure 2 Summary of all genetic alterations in the 22 SCLCs. Lines on the left of the chromosome ideogram represent a DNA loss; lines on the right represent DNA gains. Solid bars indicate a high-copy amplification. The alterations of single cases can be identified by the number on top of each line or bar, which corresponds to the case numbers of Table 1

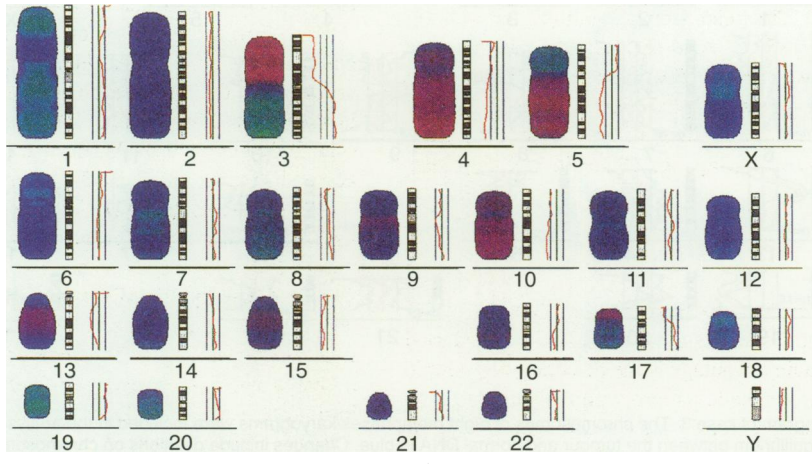

Figure 3 CGH superkaryogram of all 22 SCLCs. Typical changes are visible and include the DNA losses on chromosomes 3p, 4q, 5q, 10q, 13q and 17p and DNA over-representations of 3q and 19

DNA losses

DNA under-representations were prevalent for chromosome 3p, which was affected in all cases. In the majority of cases, the whole chromosomal arm was involved. Partial deletions occurred in three cases and the entire chromosome 3 was lost twice. The CGH and LOH analyses were consistent in each case.

Deletions on chromosome 10q were the second most frequent finding, being observed in 20 tumours. A consensus region at 10q24–qter was affected in 19 cases (86%). The LOH analysis of the two markers on chromosome 10q confirmed that this is a commonly deleted region in SCLC. Sixteen out of 19 informative cases (84%) showed allelic loss. Three examples are shown in Figure 4. The CGH data were consistent with the LOH data except for two cases. In case 12, the DNA loss by CGH could not be confirmed by LOH analysis. A second tumour (case 8, Figure 4) with no loss by CGH analysis clearly indicated a LOH for the marker D10S169 (see Discussion).

Losses on chromosomes 4q, 5q, 13q and 17p were seen in 86% (19/22) of the cases. The CGH analysis indicated the existence of two distinct regions of deletions for chromosome 5q, i.e. 5q21–22 and 5q31–qter. The LOH analysis supported the notion that in one case (no. 1) the deletion was distal to the APC locus, since the

tumour preserved heterozygosity for the markers at 5q21–22. Chromosome 15q was deleted in 12 cases (55%) and chromosome 16q in ten cases (45%).

DNA gains

DNA over-representations occurred most frequently on chromosome 3q (15/22, 68%) and 5p (12/22, 55%). These changes were consistently accompanied by an over-representation of the corresponding other chromosome arm, suggesting the formation of isochromosomes 3q and 5p respectively. High-copy amplifications were observed at 5p11 and 3q26.3–qter. DNA gains on chromosome 17q were observed in 11 cases (50%) and were accompanied by 17p loss in 10 out of 11 cases.

Over-representations were also frequent for chromosomes 19 (13/22, 59%) and 20 (11/22, 50%). DNA gains occurred in decreasing order of frequency at chromosomes 17q (11/22, 50%), 1q (8/22, 36%), 8q (7/22, 32%), 18 (7/22, 32%), 11q (6/22, 27%), 1p (6/22, 27%) and 14q (6/22, 27%). High-copy amplifications were mapped to chromosomes 1p32–36, 1q21–24, 1q41–qter, 2p23, 2q32–35, 7q11.2, 8p11, 8q21, 8q24.1, 9q21, 9q31, 11q11–14, 11q25, 13q34, 15q24–qter, 17q21, 17q25, 19p12, 19q13, 20p11, 21q22 and 22q11.

Numbers of changes

The total number of aberrations per tumour varied between 5 and 27 with a mean of 18.3. The deletions outnumbered the over-representations in 19 cases. The under-representations per tumour varied between 4 and 17 with a mean of 11.1, whereas the over-representations were between 1 and 13 with a mean of 7.1. In total, we observed 245 DNA gains and 157 DNA losses in the 22 tumours analysed. Simultaneous loss of the most frequently affected chromosomes, i.e. 3p, 4q, 5q, 10q, 13q and 17p, was found in 12 cases.

DISCUSSION

Only a few years after its initial description (Kallioniemi et al, 1992), CGH has been established as a powerful screening method in tumour genetics. Its main advantage is the detection of multiple alterations in the form of a genetic pattern within a single experiment. CGH has been used previously for the detection of aberration in SCLC (Levin et al, 1994; Ried et al, 1994). In our study, we put particular emphasis on the characterization of the deletions.

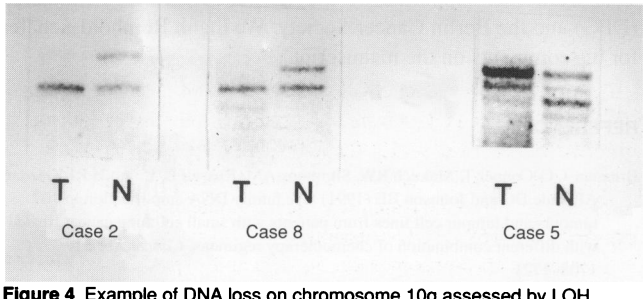

Figure 4 Example of DNA loss on chromosome 10q assessed by LOH analysis. Cases 2 and 8 were analysed for the marker D10S1213 and case 5 for the marker D10S169. The tumour sample (T) showed only one band compared with the paired normal sample (N) of the same patient as evidence for allelic loss

Deletions

Deletions on chromosome 3p were observed in all tumours. In the majority of them, the entire chromosomal arm was lost. Partial deletions in three cases were confined to two distinct non-overlapping regions at 3p11–14 and 3p21–pter. They co-localize with regions having previously been implicated as loci of potential tumour-suppressor genes (Hibi et al, 1992). The von Hippel–Lindau (VHL) gene at 3p25–26 is only rarely mutated in lung cancer cell lines (Sekido et al, 1994). Recently, the FHIT gene has been identified as a potential tumour-suppressor gene (Ohta et al, 1996). It is located at 3p14.2 and showed exonic losses in 80% of small-cell lung carcinomas (Sozzi et al, 1996), which makes it a prime candidate for lung carcinogenesis.

DNA loss of chromosome 10q was the second most frequent finding. This change has been observed previously (Ried et al, 1994; Levin et al, 1994), however at a lower incidence. The discrepancy compared with the other CGH studies might be explained by karyotyping errors. Chromosome 10 is difficult to identify on the basis of the chromosome banding by DAPI fluorescence. In particular, it might be confused with chromosome 8, which showed more frequent over-representations on the long arm in the previous studies. In our experience, it is helpful to employ a CGH analysis software that is based on a karyotyping program and allows the visualization of the different fluorescence signals during the karyotyping process. Particularly, the ratio image of the chromosomes representing the changes within a single metaphase facilitates the identification of homologous chromosomes, if they carry a characteristic change and, thus, reduces karyotyping errors.

We confirmed DNA loss on chromosome 10q by LOH analysis, which resulted in a similar incidence of deletions for this chromosomal arm. The minimal region of deletion was at 10q24–qter. Deletions on chromosome 10q have been described in gliomas (von Deimling et al, 1992; Leenstra et al, 1994), malignant menigeomas (Rempel et al, 1993), endometrial carcinomas (Peiffer et al, 1995) and melanoma (Isshiki et al, 1993). This change has been associated with tumour progression, which corresponds well with the fact that SCLC is generally an advanced and aggressive tumour. Recently, the MXI1 gene has been identified as a candidate tumour-suppressor gene in prostate cancer (Eagle et al, 1995). It is located at 10q24–25 and encodes a protein that negatively regulates the *myc* oncogene (Schreiber-Agus et al, 1995). This is intriguing, since amplification and overexpression of *myc* oncogenes have been reported in small-cell lung cancer (Wong et al, 1986; Brennan et al, 1991) and are associated with advanced tumour stages (Takahashi et al, 1989).

Candidate tumour-suppressor genes for the regions that are most often deleted include the retinoblastoma gene *Rb1* at 13q14 and *p53* at 17p13. In particular, DNA loss on chromosome 13q affected the locus of the *Rb1* gene at 13q14 in all except one case. This corresponds well with the fact that the Rb1 protein is frequently inactivated or lost in SCLC (Kelley et al, 1995). The incidence of DNA loss on chromosome 17p by CGH correlated with the frequency of mutations at the *p53* locus. Allelic loss and mutations of the *p53* gene occurred in 96% and 85% of tumours respectively, in a series of 27 patients with SCLC (Sameshima et al, 1992).

The role of *MCC* and *APC*, two tumour-suppressor genes that map to 5q21–22, in lung carcinogenesis has not yet been fully elucidated. Both genes have been implicated primarily in the formation of tumours in the gastrointestinal tract. Although the *APC/MCC* locus is frequently deleted in lung carcinomas, a

centromeric region at 5q13–14 (Wieland and Bohm, 1994) and a telomeric region at 5q33–35 (Hosoe et al, 1994) are additionally affected, suggesting that there are different tumour-suppressor genes on 5q associated with lung cancer.

A single paper reported 4q deletions in 6 of 16 (37.5%) SCLC cell lines (Sekido et al, 1993). Allelic loss on chromosome 4q has also been shown in hepatocellular carcinomas (Buetow et al, 1989; Urano et al, 1991) and bladder cancer, indicating two distinct regions (Polascik et al, 1995). We observed deletions in a centromeric region at 4q11–23 and a consensus telomeric region at chromosomal band 4q32. To our knowledge, no tumour-suppressor gene has yet been identified on this chromosome.

Copy number decreases occurred less frequently on chromosome 15q (55%) and chromosome 16q (45%). Deletions on chromosome 15q were recently observed by LOH analysis in multiple tumours, including carcinomas of the lung. They were associated with progression to metastatic stage in breast carcinomas (Wick et al, 1996).

DNA over-representation

DNA amplification, being a general characteristic of tumour progression (Brison, 1993), is a frequent finding in SCLC. We observed DNA gains in particular for chromosomes 3q and 5p. In each case, they were accompanied by a DNA loss on the other chromosomal arm. The DNA gain of an entire chromosome arm may correlate to a low-level overexpression of multiple genes that reside on this chromosome arm. However, the fact that high-copy amplifications were observed at 5p11 and 3q26.3–qter probably indicates that the activation of specific proto-oncogenes contributes to a growth advantage for cells that carry a 3q or 5p gain. DNA over-representation of 3q or amplification of the telomeric part of chromosome 3q have recently been described in head and neck squamous cell carcinomas (Speicher et al, 1995; Brzoska et al, 1995).

The single most frequent high-copy amplification was mapped to chromosome 19q13, which has been previously described (Ried et al, 1994). The *fos*-B proto-oncogene is located at 19q13.3 and constitutes a candidate gene, since it is expressed in 60% of non-small-cell lung carcinomas (Wodrich and Volm, 1993). Additionally, we observed amplifications on the short arm of chromosome 19 twice. A proto-oncogene in this region is the *rel* gene that was identified by the transfection of DNA from a human melanoma cell line into NIH3T3 mouse fibroblasts. It was mapped to 19cen–p13.2 (Nimmo et al, 1991) and was overexpressed in a small proportion of human gliomas (Watkins et al, 1994).

CGH superkaryograms in tumour classification

The sum-karyograms of the 22 SCLCs were compressed to a CGH superkaryogram (Figure 3). In this representation, the prevalent findings of the tumour class are still visible. This is promising, since it seems feasible to characterize a tumour entity or subgroup by its pattern of genetic changes, as revealed by CGH. The computer-assisted comparison of the CGH pattern of a single tumour with typical alterations of different tumour entities might become a useful adjunct in tumour classification.

The correlation between the known genetic aberrations, e.g. *Rb1* and *p53*, and the chromosomal abnormalities revealed by CGH is striking. Thus, by careful examination of the CGH patterns, it is possible to correlate certain changes with a tumour phenotype and the biological behaviour, although the causative gene defect has not yet been identified.

Multiple changes within one chromosome

We frequently observed DNA gains and losses on the same chromosome, e.g. chromosomes 2q, 3 and 8p in Figure 1A. Such a pattern has recently been observed for chromosome 12 in gliomas by molecular genetic methods (Reifenberger et al, 1995). It is consistent with a proposed model for DNA amplification by chromosome breakage (Windle et al, 1991), and CGH is an ideal tool for the detection of this type of chromosomal instability.

Amplifications might complicate the interpretation of the allelic state, since the assessment of LOH is based on the comparison of the band intensities of the two alleles. The increased intensity of one band compared with the other might actually correspond to the amplification of one allele rather than an allelic loss. The correlations between the LOH and CGH analysis in our study are in agreement with those reported in the literature (Kallioniemi et al, 1994). Additional events that might lead to contradictory results between CGH and LOH analysis are submicroscopical deletions beyond the resolution of CGH, mitotic recombination and chromosomal loss followed by reduplication. These mechanisms have been discussed previously (Ried et al, 1994).

Multiple changes per tumour

The study showed that deletions in general were more prevalent than amplifications. In particular, DNA under-representations of the frequently deleted loci on chromosomes 3p, 10q, 5q, 4q, 13q and 17p were each more prevalent than any of the observed over-representations. These loci were simultaneously affected in 12 cases. In our view, this is an indication that SCLC is actually defined by this pattern of deletions. The recent findings that the number of deletions correlates with the clinical outcome of breast cancer (Isola et al, 1995), renal carcinomas (Moch et al, 1996) and head and neck squamous cell carcinoma (Li et al, 1994) support the hypothesis that, in particular, the presence of multiple deletions determines the poor prognosis for SCLC patients.

ABBREVIATIONS

CCD, charge-coupled device; CGH, comparative genomic hybridization; FITC, fluorescein isothiocyanate; TRITC, tetrarhodamine isothiocyanate; DAPI, 4,6-diamidino-2-phenylindole dihydrochloride; LOH, loss of heterozygosity; RFLP, restriction fragment length polymorphism; SCLC, small-cell lung carcinoma; SSC, saline sodium citrate buffer.

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