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Frequent Involvement of Chromosome 3p Alterations in Lung Carcinogenesis: Allelotypes of 215 Established Cell Lines at Six Chromosome 3p Loci

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Abstract We have determined the allelotypes of 215 established lung cancer cell lines by PCR analysis at six loci on the short arm of chromosome 3 (3p): D3S3 (3p12-p13), D3S30 (3p13), D3S2 (3p14-p21.1), D3S32 (3p21), D3F15S2 (3p21), and THRB (3p24). Eighty-seven small cell lung cancer (SCLC), 93 non-small cell lung cancer (NSCLC), 6 extrapulmonary SCLC, 6 mesothelioma, and 23 normal B lymphocyte (BL) cell lines were analyzed. Low levels of heterozygosity at all six 3p loci were seen in both the SCLC and NSCLC cells. SCLC cell lines exhibited the lowest frequencies of heterozygosity at D3S3 (3%), D3S2 (3%), D3F15S2 (10%), and THRB (6%) when compared with frequencies of 8, 42, 48, and 34% at these same loci in the normal population. The lowest frequencies of heterozygosities among the NSCLC cell lines were seen at D3S3 (5%), DF15S2 (17%), and THRB (15%). Adenocarcinoma (Ad) was the only subtype of NSCLC that exhibited any heterozygosity (7%) at D3S3. In addition to D3S3, the lowest frequencies of heterozygosity were seen at D3F15S2 for Ad (9%), D3S2 for large cell carcinomas (8%), and THRB for adenosquamous (0%), bronchioloalveolar (0%), and large cell (8%) carcinomas. In summary, the 3p chromosome region near the D3S3 locus (3p12-p13) appears to be involved in all forms of lung cancer with additional involvement of regions close to the D3S2 (3p14-p21.1), D3F15S2 (3p21), and THRB (3p24) loci.

Key words: lung cancer, chromosome 3p, allelotypes, DNA, heterozygosity

Consistent loss of genetic material at specific chromosomal locations serves as a signal that the regions of loss contain tumor suppressor genes which prevent disease development [1,2]. Cytogenetic analyses on small cell lung cancer (SCLC) [3,4] and non-small cell lung cancer (NSCLC) cell lines [5,6] demonstrated that such chromosomal losses resulted from interstitial or terminal deletions or from nonreciprocal translocations. These cytogenetic studies defined regions of abnormality in lung cancer cell lines with some of the most prominent deletions occurring on the short arm of chromosome 3 (3p). Numerous molecular studies have demonstrated loss of heterozygosity (LOH) at 3p in a variety of malignancies including small cell (SCLC) and non-small cell (NSCLC) primary tumors and cell lines [7–16], renal cell carcinomas [17–19] and von Hippell Lindau disease [20], ovarian [21,22], uterine cervical [21,23], and breast carcinomas [24–26], head and neck squamous cell carcinomas [27,28] as well as in other types of cancer [2]. LOH at 3p loci also has been detected in preinvasive lesions of the bronchus [29]. Several of these cytogenetic and molecular analyses cited above included lung cancer cell lines that had been established at the NCI-Navy Medical Oncology Branch. As the published findings included only 30 of the more than 300 NCI-NMOB cell lines and in view of the importance of 3p abnormalities to lung and other cancers, we undertook a comprehensive examination of 87 SCLC and 93 NSCLC cell lines using six probes that recognize polymorphisms at 3p locations previously shown to be involved in lung cancer [7–16]. In addition, we analyzed cell lines established from 6 extrapulmonary small cell tumors, 6 mesotheliomas, and 23 EBV-immortalized peripheral blood B-lymphocytes derived from some of the patients for whom lung cancer cell lines were established. We report the results of PCR analyses that have established the 3p allelotypes

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of these 215 NCI-NMOB cell lines and show that the chromosomal region near the D3S3 locus (3p12-p13) is involved in all forms of lung cancer with additional involvement of regions surrounding the D3S2 (3p14-p21.1), D3F5S2 (3p21), and THRB (3p24) loci.

MATERIALS AND METHODS

Cell Lines

Cell lines included in this analysis were established from small cell (SCLC) and non-small cell (NSCLC) lung cancer tissue obtained from patients at the NCI-Veteran's Administration (VA) Branch prior to July, 1981 and at the Bethesda Naval Hospital after that date. Details regarding procurement of the tissues, establishment of the tumor cells in culture, and maintenance of the cell lines are detailed elsewhere in this Supplement. All cell lines were provided by Drs. Herb Oie, Ed Russell, and Sylvia Stephenson.

DNA Extractions

Genomic DNAs were extracted from cell pellets by the phenol-chloroform procedure of the ABI Model 640 Automated DNA Extractor using the protocols and reagents supplied by ABI (Foster City, CA).

PCR Analyses

PCR reactions were performed in a Perkin Elmer Cetus (Norwalk, CT) Thermal Cycler 480 following the protocols of Ganly et al. [30]. The chromosomal localizations [31–34] of the six 3p polymorphic sites we studied are shown in Figure 1. Briefly, 100–400 ng of DNA were amplified in 100 μl reaction volumes in a buffer containing 10 mM Tris, pH 8.3, 1.5 mM MgCl2, 50 mM KCl, 200 μM each dNTP, 0.2 μM each primer, and 0.4 U Taq polymerase (Promega, Madison, WI). Cycle conditions were: denaturation at 95°C for 0.5 min, annealing at 55°C for 1 min, extension at 72°C for 1 min through a total of 30 cycles. Extension following the final cycle was at 72°C for 10 min. The reaction conditions for the D3F15S2 primers were modified to an annealing temperature of 65°C. Each PCR reaction was digested overnight with the restriction enzyme specific for the amplified sequence (20 U MspI for the D3S2, D3S3, and D3S30 reactions; 20 U RsaI for the S3S32 reactions, 20 U HindIII for the D3F15S2 reactions; and 20 U EcoRI for the THRB reactions). The digested reaction products were visualized by ethidium bromide following electrophoresis in 2% agarose gels. Each reaction was performed a minimum of two times for most cell line DNAs.

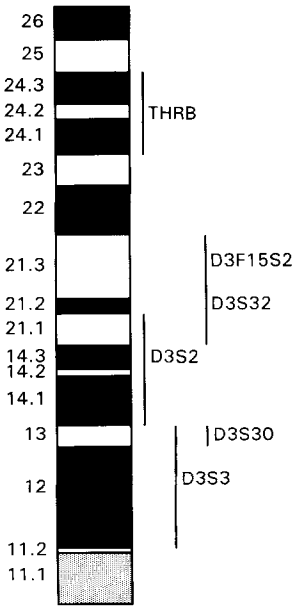

Fig. 1. Subchromosomal locations of the 3p probes used in this study.

RESULTS

Determination of Allelotypes

Figure 2 shows examples of the digestion products for the PCR fragments amplified at each of the six loci analyzed: D3S3 (Fig. 2A), D3S30 (Fig. 2B), D3S2 (Fig. 2C), D3SS32 (Fig. 2D), D3F15S2 (Fig. 2E), and THRB (Fig. 2F). The sizes of the two alleles are indicated to the left of each panel and the allelotype for each sample beneath each lane. In most instances, allelotype assignments were unambiguous. However, occasional DNA samples were encountered which did not amplify well or showed variations in digestion patterns. When this occurred, PCR reactions were repeated on the same and/or different DNA preparations. When the ratios of allele 1 to allele 2 varied among digestions with the same DNA, the appearance of restriction enzyme fragment products for allele 2 was scored as allele 2 (for examples, see Fig. 2C, lane 2, and D, lanes 1 and 3). The assignment of heterozygosity was made if allele 1 persisted at doubled restriction enzyme concentrations, in repeated digestions, or with different samples of DNA from the same cell line. Most of the digestions resulted in allelic fragments having the antici-

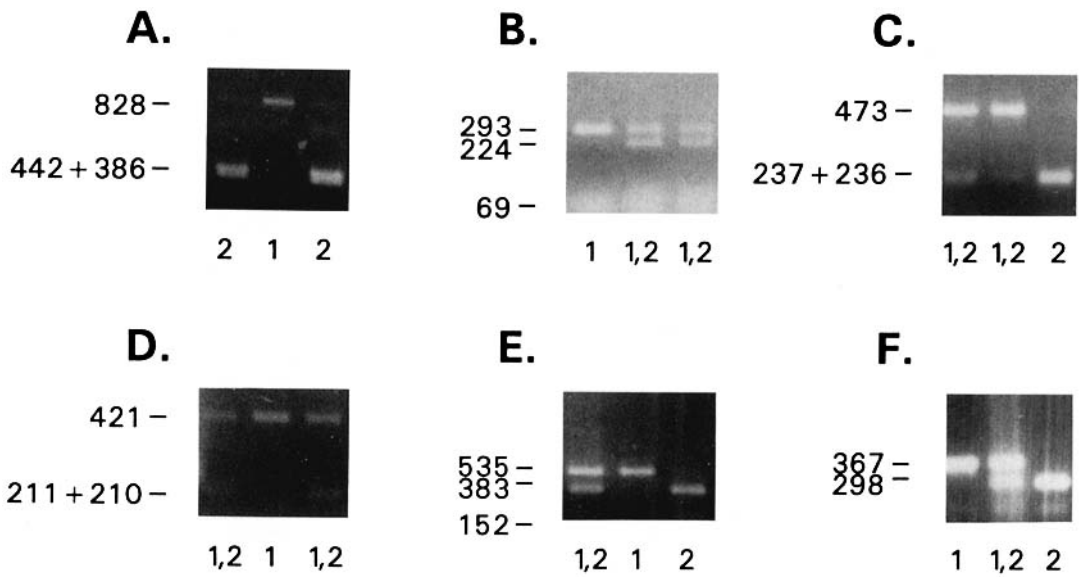

Fig. 2. Representative photographs of ethidium bromide stained gels containing restriction enzyme digestions of reaction products from PCR-amplified SCLC and NSCLC lung cancer cell lines. The samples were chosen to illustrate experimental results; different DNAs were used in each panel. The polymorphic loci analyzed were: **A:** D3S30; **B:** D3S3; **C:** D3S2; **D:** D3S32; **E:** D3F15S2; **F:** THRB. The sizes in bp to the left of each panel indicate allele 1 and 2 restriction enzyme digestion products for each reaction; the allelotypes for each DNA sample are indicated beneath each lane.

pated ratio of intensities in ethidium bromide gels.

Allelotyes of 87 SCLC Cell Line DNAs

The allelotype determinations for 87 SCLC cell line DNAs are presented in Tables IA and II. Matching BL lines were available for 12 SCLC cell lines (Table IA). Eleven of the 12 SCLC lines were informative for at least one locus and all of these lost heterozygosity for at least one locus. Three had LOH at 1 locus, 4 at 2 loci, 3 at 3 loci, and 1 at 5 loci. Highest LOHs were seen at 3p12-p13 (D3S3 = 5/5) and 3p14-p21 (D3S3 = 5/5; D3F15S2 = 4/5). The allelotypes in bold indicate agreement with published data.

Analyses of the additional 75 SCLC cell line DNAs for which corresponding BL cell lines were not available are shown in Table II. Thirty-six of these SCLC cell lines are homozygous at the five 3p loci tested, 26 are homozygous at 5 of the 6 loci tested, 11 are homozygous at 4 loci, and 2 are homozygous at 3 loci.

Allelotypes of 93 NSCLC Cell Line DNAs

The results of PCR analyses on 93 NSCLC cell line DNAs are shown in Tables IB and III. LOH for 10 NSCLC matched pairs occurred in 8 of the 10 lines that were informative for at least one locus (Table IB). Four had LOH at 1 locus, 1 at 2 loci, and 3 at 3 loci. Highest LOHs were seen at 3p13 (D3S30 = 5/5) and at 3p14-p21.1 (D3S2 = 3/4).

The analysis of the additional 83 NSCLC cell line DNAs for which paired BL lines were not available is shown in Table III. Twenty-nine of these cell lines are homozygous at all 6 loci, 24 are homozygous at 5 of the 6 loci tested, 17 are homozygous at 4 loci, 9 are homozygous at 3 loci, 3 are homozygous at 2 loci, and 1 is homozygous at 1 locus. A breakdown of the NSCLC cell line data in terms of the subtype of tumor is presented in Table IV.

Allelotypes of Extrapulmonary SCLC and Mesothelioma Cell Line DNAs

Six extrapulmonary SCLC cell lines were available for analysis (Table VA). All but one of these demonstrated heterozygosity at 1 to 5 loci [at 1, 2, 3 (in 2 cases), and 5 loci]. Of the 6 mesothelioma cell line DNAs tested (Table VB), half of them demonstrated heterozygosity [at 1, 2, and 4 loci] and the sole matched pair had LOH only at 1 of its 3 heterozygous loci. The allelotypes in bold indicate agreement with published data.

Frequencies of Heterozygosity in NCI-NMOB SCLC, NSCLC, and BL Cell Line DNAs and in the Normal Population

As presented above, immortalized BL lines were available for 23 of the NCI-NMOB tumor

TABLE IA. 3p Allelotypes of 12 Matched Pairs of SCLC and B-Lymphocyte Cell Lines*

Cell line	Histology	Subtype	D3S3	D3S30	D3S2	D3S32	D3F15S2	THRB
NCI-H128	SCLC	C	2	1	**1**	1,2	1,2	1,2
NCI-BL128	(BL1)		**1,2**	1	**1,2**	1,2	**1,2**	1
NCI-H209	SCLC	C	**2**	1,2	**1**	1	**2**	1
NCI-BL209	(BL2)		**2**	**1,2**	**1,2**	1,2	**1,2**	**1**
NCI-H1184	SCLC	C	**1**	1,2	1	1,2	**2**	1
NCI-BL1184	(BL5)		**1,2**	1,2	1,2	1,2	**2**	1
NCI-H1339	SCLC	C	**2**	**1**	**1**	1,2	**2**	1
NCI-BL1339	(BL6)		**2**	**1,2**	**1**	1,2	**2**	1
NCI-H1450	SCLC	C	**2**	**1**	**1**	1,2	**1**	1
NCI-BL1450	(BL7)		**2**	**1**	**1**	1,2	**1,2**	1
NCI-H1514	SCLC	C	2	2	1	1,2	2	1
NCI-BL1514	(BL8)		1,2	1,2	1	1,2	2	1
NCI-H1607	SCLC		2	1,2	1	2	1	1
NCI-BL1607	(BL9)		2	1,2	1	2	1,2	1
NCI-H1672	SCLC	C	2	2	1	2	2	1
NCI-BL1672			2	1,2	1	1,2	2	1
NCI-H1963	SCLC		2	1	2	2	1	1
NCI-BL1963			1,2	1,2	1,2	1,2	1,2	1
NCI-H2141	SCLC		2	1	1	2	1	2
NCI-BL2141	(BL12)		2	1	1	1,2	1,2	1,2
NCI-H2171	SCLC		2	2	1	2	2	1
NCI-BL2171			2	2	1	2	2	1
NCI-H2195	SCLC		1	2	2	2	1	1
NCI-BL2195	(BL13)		1,2	1,2	1,2	2	1	1

*Cell lines in boldface have been published previously. Allelotypes in boldface are in agreement with those published previously.

TABLE IB. 3p Allelotypes of 10 Matched Pairs of NSCLC and B-Lymphocyte Cell Lines

Cell line	Histology	Subtype	D3S3	D3S30	D3S2	D3S32	D3F15S2	THRB
NCI-H1395	NSCLC	AD	2	1	1	2	1	1
NCI-BL1395			2	1	1,2	1,2	1,2	1
NCI-H1437	NSCLC	AD	2	2	1	2	2	2
NCI-BL1437			2	2	1	2	2	1,2
NCI-H1648	NSCLC	AD	2	1	1	2	1	1
NCI-BL1648	(BL10)		2	1	1,2	2	1	1
NCI-H1770	NSCLC	**NE**	2	2	1	1,2	1,2	1,2
NCI-BL1770			2	2	1	1,2	1,2	1,2
NCI-H1819	NSCLC	AD	2	1	1	1,2	1	1
NCI-BL1819			2	1,2	1	1,2	1,2	1
NCI-H1993	NSCLC	AD	2	2	2	2	1	1
NCI-BL1993			2	1,2	2	2	1	1
NCI-H2009	NSCLC	AD	2	1	1	2	1	1
NCI-BL2009			2	1,2	1	1,2	1,2	1
NCI-H2087	NSCLC	AD	2	1	1,2	1,2	1,2	1,2
NCI-BL2087			2	1,2	1,2	1,2	1,2	1,2
NCI-H2122A	NSCLC	AD	1,2	1	1	2	1,2	1
NCI-BL2122			1,2	1	1	2	1,2	1
NCI-H2126	NSCLC	LC	2	1	1	2	1	1
NCI-BL2126			2	1,2	1,2	2	1	1,2

cell lines analyzed in this study. In order to determine whether reduction to hemizygosity had occurred among the 75 SCLC and 86 NSCLC cell lines which did not have matching BL cell lines, a different approach was used. A comparison of the frequencies of heterozygosity in the lung tumor cell line DNAs was made with the frequencies of heterozygosity found in the normal population at each of the six 3p loci (Fig. 3). The normal frequencies used in these calcula-

TABLE II. 3p Allelotypes of 75 SCLC Cell Lines*

Cell line	Histology	Subtype	D3S3	D3S30	D3S2	D3S32	D3F15S2	THRB
NCI-H60	SCLC	C	2	1,2	2	2	2	1
NCI-H64	SCLC	C	2	1	2	2	1	1
NCI-H69C	SCLC	C	2	1	2	1	1	1
NCI-H82	SCLC	V	2	1,2	2	2	2	1
NCI-H123	SCLC	C	2	1	2	2	1,2	1
NCI-H182	SCLC	C	2	1	1	1	2	ND
NCI-H187	SCLC	C	2	1	1	1,2	1,2	1
NCI-H196	SCLC	V	2	1,2	2	1	1	1
NCI-H211	SCLC	V	2	2	1	1,2	2	1
NCI-H220	SCLC	C	2	2	2	2	1	1
NCI-H250	SCLC	C	**2**	**2**	**1,2**	2	**1**	1,2
NCI-H289	SCLC	V	1	1	1	2	2	1
NCI-H345	SCLC	C	2	1	1	2	1	1
NCI-H369	SCLC	C	1,2	1	1	2	2	1
NCI-H372	SCLC	V	2	1,2	1	2	1	1
NCI-H432	SCLC	C	2	1	1	1,2	2	2
NCI-H433	SCLC	V	2	1	1	1,2	2	2
NCI-H446	SCLC	V	2	1,2	1	2	2	2
NCI-H449	SCLC	C	2	1,2	1	2	1	1
NCI-H462	SCLC	C	2	2	1	2	1	1
NCI-H524	SCLC	V	2	1	1	2	1	1
NCI-H526	SCLC	V	2	1	2	1,2	1	1
NCI-H568	SCLC	C	2	1	1,2	2	1,2	1
NCI-H620	SCLC	C	2	2	1	2	1	1
NCI-H678	SCLC	C	2	1	2	2	1	2
NCI-H689	SCLC	C	2	1	2	1,2	1	1
NCI-H711	SCLC	C	2	1,2	2	1	2	1
NCI-H719	SCLC	C	2	1	2	1	2	1
NCI-H735	SCLC	C	2	1	2	2	1	1
NCI-H738	SCLC	C	2	1	2	1	1	2
NCI-H740	SCLC	C	2	2	1	1,2	**1**	1
NCI-H748	SCLC	C	2	1,2	2	2	1	1
NCI-H774	SCLC	C	2	1	1	2	1	1
NCI-H841	SCLC	V	2	2	1	2	2	2
NCI-H847	SCLC	C	2	2	2	2	2	1
NCI-H865	SCLC	C	2	1	1	2	1,2	1
NCI-H889	SCLC	C	2	1,2	2	2	2	1
NCI-H930	SCLC	C	2	1,2	1	1,2	2	1
NCI-H1008	SCLC		1,2	1	1	2	1,2	1
NCI-H1045	SCLC	C	2	1	1	2	1	1
NCI-H1062	SCLC	C	1	1,2	1	2	1	1
NCI-H1086	SCLC		2	1	1	1,2	1	1
NCI-H1092	SCLC	C	1	2	1	2	2	1
NCI-H1105	SCLC	C	2	1	2	2	2	1
NCI-H1173	SCLC	C	2	1	**1**	1,2	1	2
NCI-H1185	SCLC	C	**2**	1,2	**1,2**	1,2	**1**	1
NCI-H1238	SCLC		2	1	1	2	2	1
NCI-H1284	SCLC	C	2	1,2	1	1,2	1	1
NCI-H1304	SCLC	C	2	1,2	1	1,2	1	1
NCI-H1417	SCLC	C	2	2	1	1	1,2	1
NCI-H1436	SCLC	C	2	1,2	1	2	2	1
NCI-H1470	SCLC		2	2	1	1	2	1
NCI-H1522	SCLC		2	1	1	1	2	1
NCI-H1618	SCLC		2	1	1	1,2	1,2	1
NCI-H1622	SCLC	C	1	1,2	1	1,2	2	1
NCI-H1628	SCLC		2	1	2	2	1	1
NCI-H1688	SCLC		2	1,2	1	2	2	1
NCI-H1694	SCLC	C	1	1,2	1	2	2	1,2
NCI-H1769	SCLC		2	2	2	1	1	1
NCI-H1788	SCLC		2	1	1	2	2	2
NCI-H1836	SCLC	C	2	1	1	2	2	1
NCI-H1876	SCLC	C	2	2	1	2	2	1
NCI-H1882	SCLC		2	2	1	2	2	1
NCI-H1930	SCLC	C	1	2	1	1,2	1	2
NCI-H1994	SCLC	C	2	1	1	2	1,2	1,2
NCI-H2028	SCLC		2	2	1	2	1	1
NCI-H2029	SCLC		2	1	1	2	1	2
NCI-H2059	SCLC	C	1	1,2	1	2	1,2	1,2
NCI-H2081	SCLC	C	2	1	1	1,2	2	1
NCI-H2107	SCLC		1,2	1	1	2	2	1
NCI-H2108	SCLC		2	1	1	2	2	1
NCI-H2196	SCLC		1	1,2	2	2	1	1
NCI-H2198	SCLC		1	1,2	2	2	1	1
NCI-H2227	SCLC		2	1	1	2	2	1
NCI-H2330	SCLC		2	1	2	1	1	1

*Cell lines in boldface have been published previously. Allelotypes in boldface are in agreement with those published previously.

TABLE III. 3p Allelotypes of 83 NSCLC Cell Lines*

Cell line	Histology	Subtype	D3S3	D3S30	D3S2	D3S32	D3F15S2	THRB
NCI-H23	NSCLC	AD	**2**	**2**	1	2	**2**	1
NCI-H125	NSCLC	ADSQ	**2**	1,2	**1,2**	1,2	**1,2**	1
NCI-H157	NSCLC	ADSQ	2	1,2	1,2	1,2	**1**	1
NCI-H226	NSCLC	SQ	**2**	**1,2**	**1**	1	2	1
NCI-H292	NSCLC	MUCEP	**2**	**1**	**2**	2	**1**	1
NCI-H322	NSCLC	BA	2	1	1	2	2	2
NCI-H324	NSCLC	AD	2	1,2	2	1	ND	1
NCI-H358	NSCLC	BA	**2**	1,2	**1**	1,2	**1,2**	2
NCI-H460	NSCLC	LC	**2**	**1**	**2**	2	**1,2**	1
NCI-H520	NSCLC	SQ	2	1	1	1	2	2
NCI-H522	NSCLC	AD	2	1	2	2	2	1
NCI-H596	NSCLC	ADSQ	2	2	1	2	2	1
NCI-H640	NSCLC	LC	2	2	1	2	2	2
NCI-H647	NSCLC	ADSQ	2	1	1	2	1	1
NCI-H650	NSCLC	LC	2	2	1	1,2	2	1
NCI-H661	NSCLC	LC	2	1,2	1,2	1,2	2	2
NCI-H676	NSCLC	AD	2	1,2	1,2	1,2	2	1
NCI-H679	NSCLC	ATYPCA	1	1,2	2	1	1	1,2
NCI-H720	NSCLC	ATYPCA	2	1	1	2	1,2	1
NCI-H726	NSCLC	AD	2	2	1	1	2	2
NCI-H727	NSCLC	CA	2	1,2	2	2	1	1
NCI-H810	NSCLC	LC	**2**	1,2	2	1	**2**	1
NCI-H820	NSCLC	BA	**2**	**1,2**	**2**	1,2	**1**	1
NCI-H835	NSCLC	ATYPCA	2	1,2	1	2	1	1
NCI-H838	NSCLC	AD	2	1	1	2	2	2
NCI-H920	NSCLC	AD	2	1	2	2	2	1
NCI-H969	NSCLC	AD	1,2	2	1	2	1	1,2
NCI-H1155	NSCLC	LC	2	1	2	1,2	1	1
NCI-H1264	NSCLC	SQ	2	**1,2**	**1,2**	2	**1,2**	1
NCI-H1299	NSCLC	LC	2	1	1	1,2	1	2
NCI-H1334	NSCLC	LC	2	1	1	1,2	1,2	1,2
NCI-H1355	NSCLC	AD	2	2	2	2	2	1
NCI-H1373	NSCLC	AD	1,2	**1,2**	**2**	1,2	1	1,2
NCI-H1378	NSCLC	SQ	1	2	1	2	2	1
NCI-H1385	NSCLC	SQ	2	1	1,2	1	1,2	1
NCI-H1404	NSCLC	BA	2	1	1	2	1	1
NCI-H1435	NSCLC	AD-NE	2	1,2	1	2	1	1
NCI-H1447	NSCLC	AD	2	2	1	2	2	2
NCI-H1466	NSCLC	AD	2	1	1	2	1	1
NCI-H1498	NSCLC	BA	1	1	1,2	2	1,2	1
NCI-H1548	NSCLC	AD	2	1,2	2	2	2	1
NCI-H1563	NSCLC	SQ	2	1	1,2	1,2	1	1
NCI-H1568	NSCLC	AD	2	2	1	2	2	2
NCI-H1570	NSCLC	LC	1	2	1	1,2	2	1
NCI-H1573	NSCLC	AD	2	2	1	1,2	2	1
NCI-H1581	NSCLC	LC	2	1	1	2	1	1
NCI-H1623	NSCLC	AD	2	2	2	1,2	2	2
NCI-H1650	NSCLC	BA	2	2	1	2	2	1
NCI-H1651	NSCLC	AD	2	1	1	2	1	1
NCI-H1666	NSCLC	BA	2	2	1,2	1,2	2	2
NCI-H1693	NSCLC	AD	2	1	1	1,2	1	1
NCI-H1703	NSCLC	SQ	2	2	1	2	1,2	1,2
NCI-H1710	NSCLC	BA	2	1,2	1	2	1	1
NCI-H1717	NSCLC	SQ	2	2	1	2	1	1
NCI-H1734	NSCLC	AD	2	1	1	2	1	2
NCI-H1755	NSCLC	AD	2	2	2	2	1	1
NCI-H1781	NSCLC	BA	2	1,2	1	1,2	2	1

Table 3 continues on next page

TABLE III. 3p Allelotypes of 83 NSCLC Cell Lines* (continued)

Cell line	Histology	Subtype	D3S3	D3S30	D3S2	D3S32	D3F15S2	THRB
NCI-H1792	NSCLC	AD	2	1	1	2	2	1
NCI-H1793	NSCLC	AD	2	1,2	1,2	1,2	2	1
NCI-H1869	NSCLC	SQ	2	1	1,2	2	2	1,2
NCI-H1915	NSCLC	LC	2	1,2	2	1,2	1	1
NCI-H1944	NSCLC	AD	2	1,2	1	2	1	2
NCI-H1975	NSCLC	AD	2	1,2	1,2	2	1	1
NCI-H2023	NSCLC	AD	2	1	1	1,2	1	1
NCI-H2030	NSCLC	AD	2	2	1	2	2	1
NCI-H2066	NSCLC	ADSQ	2	1,2	1	1,2	1	1
NCI-H2073	NSCLC	AD	2	2	2	2	1	1
NCI-H2077	NSCLC	AD	2	1	1	2	1	1
NCI-H2085	NSCLC	AD	2	1,2	1,2	1,2	1	1
NCI-H2086	NSCLC	AD	2	1,2	1,2	1,2	1	1
NCI-H2106	NSCLC	NE	2	2	1	1,2	1,2	1,2
NCI-H2110	NSCLC		1,2	2	2	2	2	1
NCI-H2135	NSCLC		1,2	1	1,2	2	1,2	1,2
NCI-H2170	NSCLC	SQ	2	2	1	1,2	2	1
NCI-H2172	NSCLC		1	2	1,2	1,2	1	1
NCI-H2250	NSCLC	AD	2	1	1	2	1	1,2
NCI-H2558	NSCLC	AD	2	1	1	2	1	1,2
NCI-H2291	NSCLC	AD	2	1	1,2	1,2	2	2
NCI-H2342	NSCLC	AD	2	2	1	2	2	1
NCI-H2347	NSCLC		2	1	2	1	2	1
NCI-H2374	NSCLC	AD	2	2	1,2	2	1,2	1
NCI-H2405	NSCLC	AD	2	1	1	1	2	1,2
NCI-H2427	NSCLC	AD	2	1,2	1,2	1,2	1,2	1,2

*Cell lines in boldface have been published previously. Allelotypes in boldface are in agreement with those published previously.

TABLE IV. Heterozygosity in NCI-NSCLC Cell Lines by Subtype*

NSCLC subtype	D3S3 (%)	D3S30 (%)	D3S2 (%)	D3S32 (%)	D3F15S2 (%)	THRB (%)
Ad (45)	3/45 (7)	10/45 (22)	9/45 (20)	13/45 (29)	4/45 (9)	7/45 (16)
AdSq (5)	0/5 (0)	3/5 (60)	2/5 (40)	3/5 (60)	1/5 (20)	0/5 (0)
Sq (10)	0/10 (0)	2/10 (20)	4/10 (40)	2/10 (20)	3/10 (30)	2/10 (20)
LC (12)	0/12 (0)	3/12 (25)	1/12 (8)	7/12 (58)	2/12 (17)	1/12 (8)
BA (9)	0/9 (0)	4/9 (44)	2/9 (22)	4/9 (44)	2/9 (22)	0/9 (0)

*Twelve cell lines are not included due to low numbers of a subtype or the subtype was not determined.

TABLE VA. 3p Allelotypes of 6 Extrapulmonary Cell Lines*

Cell line	Histology	Subtype	D3S3	D3S30	D3S2	D3S32	D3F15S2	THRB
NCI-H510A	SCLC	EXP	2	1	1	2	**1,2**	1
NCI-H660	SCLC	EXP	2	**1,2**	1,2	1,2	**1,2**	1,2
NCI-H1048	SCLC	EXP	2	**1,2**	1,2	2	1,2	1
NCI-H1341	SCLC	EXP	**1,2**	2	1	2	**1,2**	1
NCI-H1870	SCLC	EXP	2	1	1	2	1	1
NCI-H1926	SCLC	EXP	2	1,2	2	1,2	1,2	1

*Cell lines in boldface have been published previously. Allelotypes in boldface are in agreement with those published previously.

TABLE VB. 3p Allelotypes of 6 Mesothelioma Cell Lines and 1 Matched BL Line

Cell line	Subtype	D3S3	D3S30	D3S2	D3S32	D3F15S2	THRB
NCI-H28	ME	2	2	1	2	1	1
NCI-H290	ME	2	1	1	2	2	1
NCI-H513	ME	2	1,2	1,2	1,2	1,2	1
NCI-H2052	ME	1	1,2	1,2	2	2	1
NCI-BL2052		1	1,2	1,2	2	2	1,2
NCI-H2058	ME	2	2	1	2	1	1
NCI-H2373	ME	2	2	1	1,2	1	1

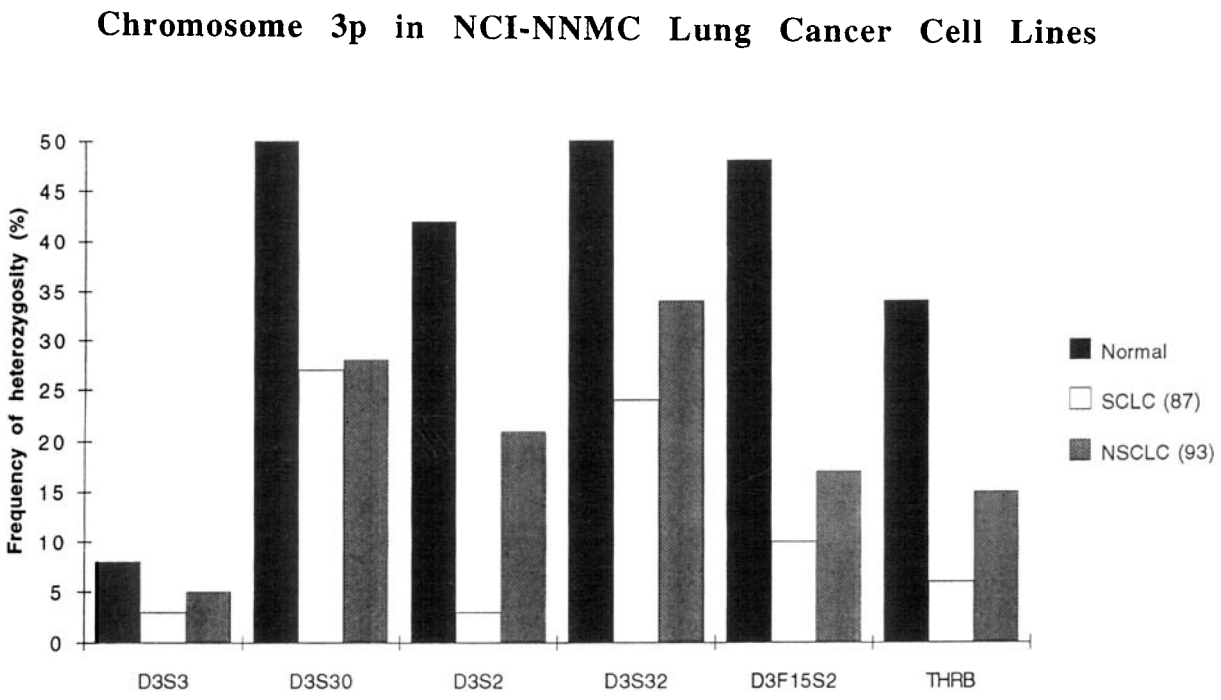

Fig. 3. Comparative frequencies of heterozygosity determined by PCR analyses for 87 SCLC (open bars) and 93 NSCLC (hatched bars) cell line DNAs. The frequencies of heterozygosity were determined from the allelic determinations in Tables IA,B, II, and III. These frequencies are compared with frequencies of heterozygosity for the normal population (black bars) as reported in the literature (Table VI).

tions were those reported in the literature [15,27]. [The numbers of extrapulmonary SCLC and mesothelioma cells analyzed were too small (6 each) to be subjected to this type of analysis.]

Allelic Frequencies in the NCI-NMOB SCLC and NSCLC Cell Line DNAs

To determine if there was any preferred loss of one allele over the other at any of the six loci studied, we compared allelic frequencies of the SCLC and NSCLC cell line DNAs with those determined for the normal population. The ratios for normal individuals were those published for each locus [15,35–38]. As can be seen in Table VI, no significant differences in allelic ratios were seen in this lung tumor cell line population, suggesting random allelic loss at the tested loci.

Discrepancies of PCR Allelotypes With Previously Published Data

We noted several discrepancies when we compared our PCR-generated data with allelotypes published in 1987 [7,9] and 1990 [15]. For example in Table IA, our SCLC analysis shows differences for NCI-H128 at D3S3 (allele 2 as opposed to allele 1 previously determined [9]) and D3F15S2 (alleles 1, 2 as opposed to allele 2

TABLE VI. Allelic Frequencies for Chromosome 3p Loci

Locus	Location	Enzyme	Alleles (a;b)	Allelic frequencies		
				Normal	SCLC	NSCLC
D3S3	3p12-p13	MspI	828;442+386	0.04:0.96[b]	0.16:0.84	0.10:0.90
D3S30	3p13	MspI	293;224+69	0.51:0.49[a]	0.60:0.40	0.54:0.46
D3S2	3p14-p21.1	MspI	473;237+236	0.70:0.30[b]	0.69:0.31	0.64:0.36
D3S32	3p21	RsaI	421;211+210	0.47:0.53[c]	0.31:0.69	0.32:0.68
D3F15S2	3p21	HindIII	535;383+152	0.41:0.59[d]	0.45:0.55	0.52:0.48
THRB	3p24	EcoRI	367;298+69	0.78:0.22[e]	0.82:0.17	0.72:0.28

[a]Nakamura et al. [35].
[b]Barker et al. [36].
[c]Fujimoto et al. [37].
[d]Latif et al. [27].
[e]Middleton et al. [38].

[9]). One internal discrepancy for the same cell line is seen for THRB where the tumor cell line has both alleles 1 and 2 whereas NCI-BL128 has only allele 1. The allelotype of BL128 matches that published [9]. Similar discrepancies appear in cell line NCI-H209 at D3S3 (allele 2 vs. allele 1 [9]) and D3F15S2 (allele 2 vs. allele 1 [9]). Moreover, LOH [15] was noted at D3S30 but was not detected in this analysis. These data suggest that the current NCI-H128 and NCI-H209 cell lines are not the same lines that were analyzed in 1987. With regard to NCI-BL209, both this analysis and Brauch et al. [15] detect homozygosity at D3S2 and D3F15S2 in contrast to an earlier report [9]. Likewise, we find NCI-BL1184 to be heterozygous at D3S2 and homozygous at D3S30 in conflict with the earlier results [9] and this D3S30 analysis indicates a *gain* of heterozygosity in the NCI-H1184 tumor DNA which is not likely to occur. Two discrepancies are noted in the data presented in Table II: NCI-H847 at D3F15S2 (allele 2 vs. allele 1 previously [11]) and NCI-H1185 at D3S30 (heterozygous versus homozygous previously [15]). (D3S32 and THRB allelotypes cannot be compared with data in the literature since we examined different polymorphisms at these loci.)

Discrepancies in the NSCLC allelotyping with previously reported determinations [15] are seen in Table III at D3S2 [NCI-H157 is heterozygous], D3S3 [NCI-H1373 is heterozygous], D3S30 [NCI-H125, -H157, -H358, and -H810 are heterozygous] and D3F15S2 [NCI-H226 and -H1373 are homozygous.] There were no discrepancies in our extrapulmonary SCLC cell line data with published allelic determinations [39].

DISCUSSION

Chromosome 3p allelotype determinations for 215 lung tumor and related cell lines established at the NCI-Navy Medical Oncology Branch are presented. The data constitute a "3p profile" of these cell lines which can be used in conjunction with other data in this Supplement to identify a particular cell line. Our PCR data show high levels of homozygosity (or reduction to hemizygosity) at all six 3p loci examined when compared with frequencies of heterozygosity determined for the normal population. The most dramatic decreases are seen at D3S3, D3S2, D3F15S2, and THRB. Homozygosity is greater in the SCLC cell lines than in the NSCLC cell lines at all loci studied. These results confirm and strengthen previous cytogenetic and LOH analyses that indicate the importance of loss of chromosome 3p regions in the development of SCLC and NSCLC cancers [3–17].

Marked homozygosity was noted for both SCLC and NSCLC with the most striking differences from the normal frequencies noted at D3S3, D3S2, D3F15S2, and THRB in SCLC and at D3S3, and to a lesser extent D3S2, D3F15S2, and THRB, in NSCLC. Adenocarcinomas are primarily affected at D3S3, D3F15S2, and THRB and less at D3S30, D3S2, and D3S32. Adenosquamous carcinomas are most affected at D3S3 and THRB and less at D3F15S2; D3S30, D3S2, and D3S32 appear to be uninvolved in this subtype. D3S3 is most involved in squamous carcinomas with slight involvement of most of the other loci except D3S2 which appears to be uninvolved. D3S3, D3S2, and THRB are most involved in large cell carcinomas with D3S30

and D3F15S2 contributing slightly and D3S32 uninvolved. Bronchiolo-alveolar carcinoma shows involvement of only D3S3 and THRB with some contribution from D3S2 and D3F15S2; D3S30 and D3S32 appear to be uninvolved.

The D3S3 locus had the highest level of homozygosity in the SCLC and NSCLC cell lines and only adenocarcinomas exhibited heterozygosity (7%) at D3S3. This proximal chromosomal region (3p12-p13) is within reported homozygous deletions in SCLC [40,41]. The D3F15S2 polymorphic probe maps to 3p21-3p23 [32], a region at which several homozygous deletions in lung cancers and cell lines have been reported [42,43] and to which several candidate 3p tumor suppressor genes have been localized [44,45]. We experienced some difficulty in PCR amplification of several cell line DNAs at the D3F15S2 locus although these same DNAs amplified well at the other 3p loci, indicating that there might be specific features within the D3F15S2 locus which have been altered in these cell lines. The nature of such alterations, if they do exist, will become apparent as this locus is characterized in greater detail and the identity of the tumor suppressor gene(s) thought to lie in the region is established.

An expansion of this study to include an additional 43 primer pairs specific to other 3p loci resulted in the detection of a homozygous deletion at 3p21.3 in an SCLC cell line (NCI-H740) established at the NCI-NMOB [43].

The necessity for this "3p profile" is underscored by the discrepancies detailed in Results. Whether these discrepancies are the result of (1) genetic changes that have occurred during long-term passage of these cell lines in tissue culture; (2) the outgrowth of one subclone in a culture having mixed phenotypes and karyotypes [46]; (3) the loss of a restriction site due to random mutation; (4) error in interpretation of LOH or PCR data; (5) accidental switching or cross-contamination of cell lines and/or DNA samples; or (6) other uncontrollable factors encountered during growth and maintenance of these cultures for periods up to 18 years can be determined only by retrieving and analyzing very early passages of the cell lines in which discrepancies have occurred. The data we present were generated on cell lines grown during the period 1990–1992. These PCR profiles will be useful in identifying specific cell lines and in establishing the regions of 3p important in SCLC and NSCLC carcinogenesis.

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