Pergamon

European Journal of Cancer Vol. 32A, No. 2, pp. 335–341, 1996

Printed in Great Britain
0959-8049/96 $15.00+0.00

0959-8049(95)00535-8

Original Paper

Frequent *TP53* Gene Alterations (Mutation, Allelic Loss, Nuclear Accumulation) in Primary Non-small Cell Lung Cancer

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Mutations of the *TP53* tumour suppressor gene have been reported for many human cancers. A variety of *TP53* mutations have also been reported for both primary non-small cell lung cancer (NSCLC) and associated metastases. To assess the pathogenetic significance of *TP53* gene alterations in NSCLC, 24 paired samples of primary NSCLC and the corresponding normal lung tissue were analysed for mutations of the *TP53* gene (exons 5–8) using exon-specific PCR, single-strand conformation polymorphism PCR (SSCP-PCR) and direct DNA sequencing; for p53 protein accumulation by immunohistochemistry and for 17p allelic loss using restriction fragment length polymorphism (RFLP) probes on Southern blots and amplified fragment length polymorphism-PCR. *TP53* point mutations were observed in 9/24 (38%) tumours encompassing a total of 14 mutations. Two tumours displayed the same double mutation while a third harboured four different mutations. Seventeen of 24 NSCLCs (71%) overexpressed p53 protein and all 17 immunopositive tumours (100%) showed a mutation and/or allelic loss at the *D17S30* locus. Of the 17 NSCLCs informative at the *DS17S30* locus, 10 (59%) showed allelic loss, of which five (50%) were also mutated on the remaining *TP53* allele. These results suggest that *TP53* gene alterations are involved in the pathogenesis of primary NSCLC and that such alterations may serve a selective role in the development of NSCLC by diminishing the apoptotic potential of bronchial epithelial cells heterozygous for a *TP53* point mutation. This may also explain the accumulation of multiple *TP53* point mutations in 3/24 of our NSCLC samples.

Key words: p53 mutations, p53 accumulation, D17S30 allelic loss, non-small cell lung cancer
Eur J Cancer, Vol. 32A, No. 2, pp. 335–341, 1996

INTRODUCTION

CANCER OF THE lung is the most frequent cause of cancer-related death in the Western world. Approximately 90% of primary lung cancer patients will succumb to their disease within one year of diagnosis [1]. The two major histological forms of lung cancer are non-small cell lung cancer (NSCLC), which represents 75% of cases at presentation, and small cell lung cancer (SCLC) which represents 25% [2]. The investigation of molecular events associated with lung cancer development has revealed multiple genetic alterations which are consistent with a multistep model of carcinogenesis. Accumulating evidence suggests that genetic alterations of both dominant oncogenes and tumour suppressor genes are obligatory events in the malignant transformation of normal bronchial epithelium. In this context, mutations affecting the *TP53* gene could contribute to this process [3–8].

Although similar to the multistep model of carcinogenesis proposed for colorectal cancer, the temporal sequence of these genetic alterations may differ in lung cancer. In colorectal, thyroid and ovarian cancer, *TP53* mutations appear during tumour progression [9–11]. In brain, adrenal gland, oesophageal and breast cancer, *TP53* mutations are among the first detectable genetic alterations [12–15]. Allelic loss or imbalance at 17p13 is frequently observed in lung cancer, with approximately 80% of tumours displaying concordant loss of one *TP53* allele and a point mutation on the remaining *TP53* allele [16–18]. In some studies the presence of *TP53* alterations is not associated with a poor prognosis or tumour stage [19, 20] whereas in others, *TP53* alterations have been associated with smoking history, lymph node metastases, advanced tumour stage and poor prognosis [18, 21–23]. Studies of preneoplastic bronchial lesions have also suggested that *TP53* alterations may be an early event in lung cancer development [24–26].

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Revised 9 Aug. 1995; accepted 23 Aug. 1995.

In order to assess whether alterations to the *TP*53 gene contribute to the pathology of NSCLC, we analysed tissue from primary, non-pretreated NSCLC. Five regions of the *TP*53 gene are frequently modified in human tumours (five "hot-spot regions", HSRs) and contain the conserved amino acids most often affected by mutation. The five HSRs comprise *TP*53 exons 5 to 8 and account for 70–80% of mutations detected to date. We examined *TP*53 point mutations in these HSRs with respect to allelic loss at the *D17S30* locus (17p13), the intracellular accumulation of the p53 oncoprotein and associated clinical parameters in order to evaluate the relationship of *TP*53 genetic changes to the development of primary NSCLC.

MATERIALS AND METHODS

Tissues

Genomic DNA was prepared from NSCLC tissue from 24 NSCLC patients who underwent complete resection of the primary tumour. No patients in the study group had received previous chemotherapy, immunotherapy or radiation therapy treatments. Samples ($\approx$ 1 cm^3) of tumour tissue and adjacent non-neoplastic lung tissue were removed and processed for genomic DNA isolation. Portions of the tumour tissue were also removed for histological examination and classification.

DNA extraction and analysis

Genomic DNA was extracted from both tumour and normal lung tissue according to a previously published method [27]. Of the genomic DNA, 10 μg was digested with the appropriate restriction enzyme, subjected to electrophoresis in 0.8% agarose gels, transferred to Hybond-N Nylon membranes (Amersham, U.K.) and incubated for 2 h at 80°C. The membranes were then prehybridised and hybridised with α-^{32}P-labelled random-primed pYNZ22.1 RFLP probe in Church hybridisation buffer [28]. After hybridisation at 65°C for 18 h, the membranes were washed sequentially for 15 min at room temperature in 2× SSC : 1% SDS : 1 mM EDTA, at 65°C in 2× SSC : 0.1% SDS and at 65°C in 0.1× SSC : 0.1% SDS before autoradiography with Kodak X-OmatAR film. 1× SSC comprises 150 mM sodium chloride and 15 mM sodium citrate pH 7.0.

The pYNZ22.1 probe (*D17S30*) was obtained from the American Type Culture Collection (Rockville, Maryland, U.S.A.) and detects a 10 allele restriction fragment length polymorphism at 17p13.3 using either PstI, HinfI, MspI or TaqI [29]. All these enzymes were used in our analysis of 17p13 allelic loss in NSCLC.

Table 1. *Allelic loss at* D17S30 *immunohistochemistry and* TP53 *mutations in NSCLC*

Tumour	LOH*	IHC†	*TP*53‡	Tumour Type§	Stage‖	Mutation¶	Amino acid change
12	del	+2	5-158	SQC	T2N1-II	CgC>CcC	Arg>Pro
16	del	+3	5-167/168	SQC	T2N2-III	Cag.cAC>CAC	Gln.His>His (3bp deletion between codons 167 and 168)
28	ndel	+2	8-276	SQC	T2N0-I	GcC>GaC	Ala>Asp
			8-278			CcT>CgT	Pro>Arg
30	ndel	+3	wt	SQC	T1N0-I		
50	del	0	wt	SQC	T2N2-III		
54	del	+1	8-278	SQC	T2NO-I	CcT>CgT	Pro>Arg
58	ndel	+3	5-161	SQC	T4N2-III	GcCA>GCA	Ala>Ala (TGA Stop codon 23bp from deletion site)
			5-173			G.TgAG>G.TAG	TAG Stop codon generated at deletion site
			5-175			CGc>CGg	Arg>Arg
			5-177			CcC>CtC	Pro>Leu
72	ni	0	wt	SQC	T2N0-I		
88	ndel	+3	wt	SQC	T1N2-II		
106	del	+3	wt	SQC	T3N1-III		
108	ni	0	wt	SQC	T1N0-I		
116	ni	+3	5-175	SQC	T2N1-II	CgC>CaC	Arg>His
118	ndel	+2	wt	SQC	T1N1-II		
18	del	+2	wt	LLC	T2N1-II		
20	del	+3	5-163	LLC	T4N2-III	TaC>TgC	Tyr>Cys
26	ni	+2	8-276	LLC	T2N2-III	GcC>GaC	Ala>Asp
			8-278			CcT>CgT	Pro>Arg
56	del	+3	8-286	LLC	T1N0-I	gAA>aAA	Glu>Lys
60	del	0	wt	LLC	T2N1-II		
86	ndel	+3	wt	LLC	T1N2-II		
96	del	0	wt	LLC	TxNx		
114	ndel	+1	wt	LLC	T2N2-III		
80	ni	0	wt	ADC	TxNx		
112	ni	0	wt	ADC	T1N0-I		
122	ni	+1	wt	ADC	TxNx		

**D17S30* allelic loss: ndel, heterozygous-not deleted; del, heterozygous-deleted; ni, homozygous-not informative. †Percentage of αp53-positive cells: 0, < 5%; +1, > 10%; +2, > 50%; +3, > 90%. ‡Alterations to *TP*53 exons 5, 6, 7 or 8: (wt) wild-type *TP*53 sequence; numbers indicate the *TP*53 exons and codons with mutations. §Tumour type: ADC, adenocarcinoma; LLC, large cell carcinoma; SQC, squamous cell carcinoma. ‖Tumour size, node involvement and stage. ¶Base pairs affected by mutation are indicated by lower case letters.

pYNZ22 amplified fragment length polymorphism (AFLP) PCR

Normal lung tumour tissue pairs, which gave no clear signals on Southern blots, were subsequently analysed using a PCR-based method described by Batanian and colleagues [30] which detects a polymorphic repeat unit within the region detected by the pYNZ22.1 probe (*D17S30*) at 17p13.3. Using this method, allelic loss at 17p13 in these samples could be determined.

*TP*53 *PCR and DNA sequencing*

A DNA fragment encompassing *TP*53 exons 5–8 was amplified from genomic DNA by PCR using the intron-specific primers described by Baker and colleagues [31]. The PCRs were performed in a 100 μl volume using 200 ng genomic DNA, 0.2 μM of each primer, 5% DMSO, 0.05% W1, 1.25 mM $MgCl_2$, 200 μM dNTPs and 1U Taq polymerase (Gibco BRL, Basel, Switzerland) in the supplied buffer. The PCR conditions were as follows: 10 min at 95°C followed by 40 cycles of 1 min at 95°C, 2 min at 55°C and 4 min at 70°C with a final extension step of 10 min at 70°C. A 10 μl aliquot of this 100 μl primary PCR was used as template for a second exon-specific PCR. The PCR conditions were the same as above apart from replacement of the initial 10 min at 95°C denaturation step with one cycle of 5 min at 95°C, 3 min at 55°C and 3 min at 70°C. The *TP*53 exon fragments were purified on 1% NuSieve agarose gels (FMC) and Genecleaned® (Bio 101) prior to sequencing with exon-specific primers and the fmole® cycle sequencing kit (Promega, Wallisellen, Switzerland). *TP*53 mutations were confirmed by at least three independent genomic PCRs followed by exon-specific PCR and cycle sequencing.

TP53 *exon cloning and sequencing*

*TP*53 exon-specific PCR fragments were gel-purified as described above and cloned into the pGEM-T vector (Promega) according to the manufacturer's instructions. Insert-containing plasmids were isolated and confirmed by vector-PCR using insert (exon)-specific PCR primers [32]. Five independent clones were sequenced from each sample using the Sequenase kit® (USB/Amersham) and the plasmid-specific T7 sequencing primer.

Single-strand conformation polymorphism (SSCP) PCR

Exon-specific PCR (100 μl) was performed on NSCLC samples displaying single and double point mutations. These PCR products were gel-purified, Genecleaned® and resuspended in 40 μl 1 × TE buffer. Of this preparation 1 μl was used as a template in a second exon-specific PCR (10 μl). In addition to the template, PCRs contained 0.2 μM of each primer, 5% DMSO, 0.05% W1, 1.25 mM $MgCl_2$, 100 μM dATP/dGTP/dTTP, 2 μM dCTP, 0.5 μCi α^{32}P dCTP and 0.5U Taq polymerase (Gibco BRL) in the supplied buffer. The PCR conditions were 4 min at 93°C followed by 20 cycles of 1 min at 93°C, 1 min at 50°C and 2 min at 70°C with a final extension step of 10 min at 70°C.

SSCP analysis was performed as described by Orita and associates [33] with the following modifications. PCR samples (2 μl) were diluted 100-fold in 95% formamide, 20 mM EDTA, 0.05% Bromophenol Blue and 0.05% xylene cyanol, heated at 95°C for 10 min, "quick-cooled" on ice and applied (2 μl per lane) to a 0.5× MDE® gel (AT Biochem Inc, Malvern, Pennsylvania, U.S.A.) containing 1× TBE and 10% glycerol. Gels were electrophoresed in 1× TBE at room temperature at 3 W for 15 h. The gel was subsequently dried and exposed to Kodak X-OmatAR autoradiography film with intensifying screens at room temperature for 5–18 h.

p53 immunohistochemistry

Paraffin sections (4 μm) of both normal lung and NSCLC tissue from the same patient were examined for p53 protein expression using a rabbit polyclonal antibody (OPA 28/2, Medac, Zurich, Switzerland) with specificity for both the wild-type and mutant forms of the p53 protein. Samples scored as positive for p53 expression exhibited intense nuclear staining in more than 5% of the tumour epithelium but not in adjacent normal epithelial or stromal tissue.

RESULTS

Mutations affecting the TP53 *coding sequence*

24 NSCLC cases were analysed for mutations in the 5 HSRs of the *TP*53 gene (exons 5–8). Exon-specific PCR and direct DNA sequencing were performed from three independent *TP*53 (exon 5–8) genomic DNA PCRs and subsequent exon-specific PCRs from each NSCLC to ensure that the results were not due to sample cross-contamination or the generation of PCR artefacts from multiple rounds of PCR. As shown in Table 1, 38% (9/24) of the NSCLC cases studied harboured point mutations in exon 5 (8/14) or 8 (6/14) of the *TP*53 gene. One "in frame" 3bp deletion, two 1bp deletions and 11 point mutations (seven transversions and four transitions) were detected in the *TP*53 coding sequence. Of

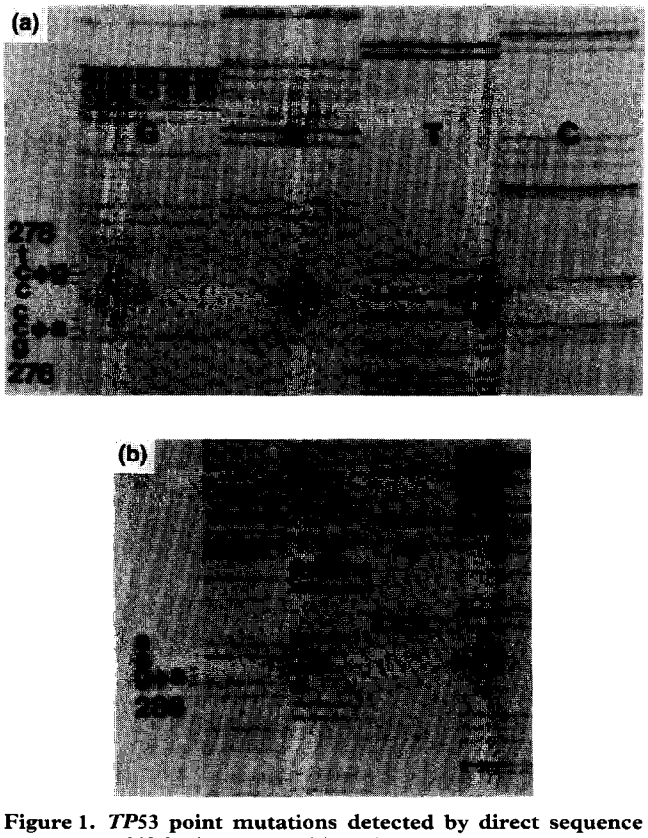

Figure 1. *TP*53 point mutations detected by direct sequence analysis in NSCLC. (a) Double point mutations detected in exon 8. (b) Single point mutation detected in exon 8. The wild-type *TP*53 sequence is given with an arrow indicating the nucleotide change detected in the NSCLC sample. The sequencing strategy for detecting *TP*53 point mutations is as described by Chiba and associates [16].

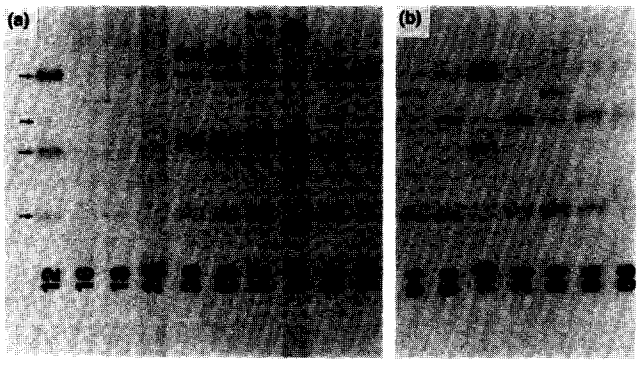

Figure 2. SSCP-PCR analysis of exon 8 point mutations in NSCLC. (a) SSCP-PCR analysis of NSCLCs displaying no mutations (nos. 12, 16, 18, 20, 58, 116), single point mutations (nos. 54, 56) or double point mutations (nos. 26, 28) in the *TP*53 exon 8 sequence. Arrows indicate the position of the exon 8 "wild-type allele conformers" in NSCLCs displaying no point mutations by direct sequence analysis. Bands migrating at positions other than those indicated by the arrows are presumed to be exon 8 "point mutant allele conformers". (b) SSCP-PCR analysis of normal lung NSCLC tissue pairs (nos. 25/26, 55/56) for germline or somatic *TP*53 point mutations with control NSCLCs displaying no mutation (nos. 58, 116) and single mutation (no. 54) in exon 8 of the *TP*53 gene.

11 point mutations, nine were at G:C and two were at A:T residues. No mutations were detected in exons 6 or 7 of the *TP*53 coding sequence. An example of the sequence analysis for exon 8 is shown in Figure 1.

Three NSCLCs with multiple point mutations were also observed (no. 26, no. 28 and no. 58). Since none of these tumours showed allelic loss at 17p13 (Table 1), inactivation of *TP*53 could be due to the mutation of each *TP*53 allele. This possibility was examined by exon-specific SSCP-PCR analysis of the appropriate NSCLC samples. As shown in Figure 2a, NSCLC samples displaying double point mutations in exon 8 (no. 26 and no. 28) also displayed two bands of altered mobility in addition to the wild-type *TP*53 exon 8 bands. This suggested that each of the *TP*53 exon 8 alleles carried a point mutation. These point mutations were also shown to be somatic by comparing the normal lung and NSCLC tissue pairs by exon-specific SSCP-PCR (Figure 2b, no. 25/26 and no. 55/56) and subsequent sequence analysis. However, NSCLCs with single exon 8 point mutations (Figure 2A, no. 54, and no. 56) also displayed SSCP banding patterns similar to those for the NSCLCs with double exon 8 point mutations. To assess whether the observed point mutations occurred on one or both of the exon alleles, the exon-specific PCR products from these NSCLCs were cloned and sequenced. As shown in Figure 3a, NSCLC 26 harboured both *TP*53 mutations on one exon 8 allele, as did NSCLC 28 (data not shown). In addition, NSCLC 58 carried a single point mutation on one exon 5 allele and three mutations on the other (Figure 3b). Although three point mutations are present on this allele, the significant change is the single base pair deletion at codon 161 which results in a TGA stop codon 23 base pairs downstream from the deletion site (Figure 3c). All the described mutations were detected in either exon 5 or exon 8 of the *TP*53 gene.

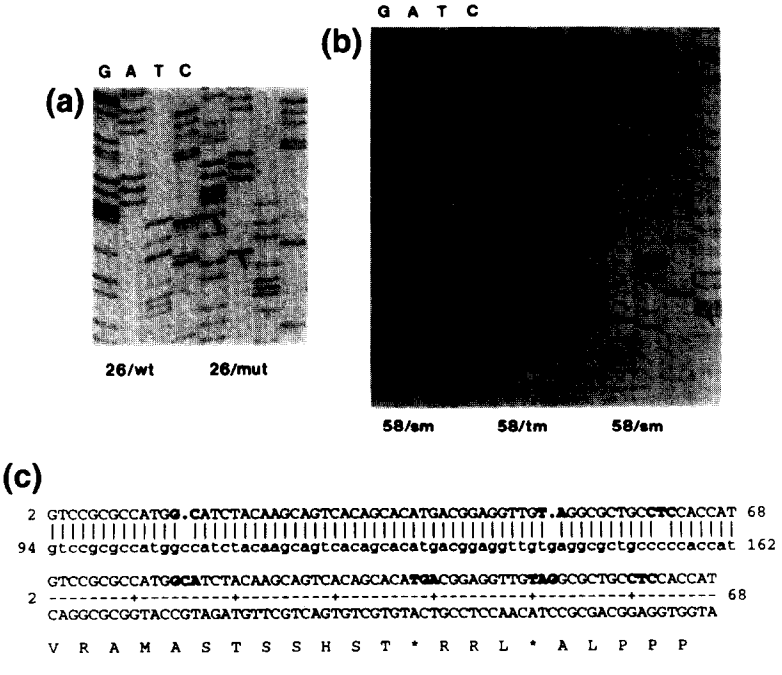

Figure 3. Sequence analysis of cloned exon 5 or 8 alleles for NSCLCs displaying multiple *TP*53 point mutations. (a) Sequence analysis of cloned exon 8 PCR products from NSCLC 26. Arrows indicate the position of mutations (codons 276, 278) in one exon 8 allele clone from NSCLC 26 (26/mut) compared with the non-mutant sequence of another NSCLC 26 exon 8 allele clone (26/wt). (b) Sequence analysis of cloned exon 5 PCR products from NSCLC 58. Arrows indicate the position of mutations in the three exon 5 allele clones (58/sm, codon 175; 58/tm, codon 177) and asterisks (*) the position of single nucleotide deletions (codons 173, 161) in one of the exon 5 allele clones (58/tm). (c) Nucleotide sequence comparison (BESTFIT) and predicted amino acid sequence (MAP) of NSCLC 58/tm exon 5 and wild-type *TP*53 exon 5. Mutation sites, deletion sites and stop codons are indicated in bold type.

Allelic loss at D17S30 (17p13)

Genomic DNA of 24 NSCLC cases and matched normal lung tissue from the same patients were subjected to allelic loss analysis using either genomic DNA Southern blots with the polymorphic pYNZ22 probe (Figure 4a) or an AFLP-PCR protocol for pYNZ22 allelic loss (Figure 4b). Of the patients analysed, 71% (17/24) were constitutionally heterozygous (informative) for the alleles detected by the pYNZ22 RFLP probe. Of these informative cases, 59% (10/17) displayed loss of one of the two pYNZ22 alleles.

Immunohistochemical analysis of p53 expression

Immunohistochemical analysis of p53 protein accumulation in the 24 NSCLC cases was performed with a rabbit polyclonal antiserum specific for both mutant and wild-type forms of human p53. Of the NSCLCs, 71% (17/24) displayed nuclear accumulation of the p53 protein. Although the staining pattern was invariably nuclear, some cases did exhibit additional weak perinuclear staining. Immunopositive staining for p53 was not observed in the surrounding non-neoplastic epithelial or stromal tissue (Figure 5). All (9/9) of the NSCLC cases exhibiting a *TP*53 exon mutation were also immunopositive (Table 1).

Association between TP53 alterations and clinical parameters

A correlation was observed between *TP*53 mutations and p53 protein accumulation (Fisher's exact test: $P = 0.022$). No significant correlations were observed between *TP*53 mutation and tumour stage (Fisher's exact test: $P = 0.68$), allelic loss and tumour stage (Fisher's exact test: $P = 0.61$) or p53 nuclear accumulation and tumour stage (Fisher's exact test: $P = 0.18$).

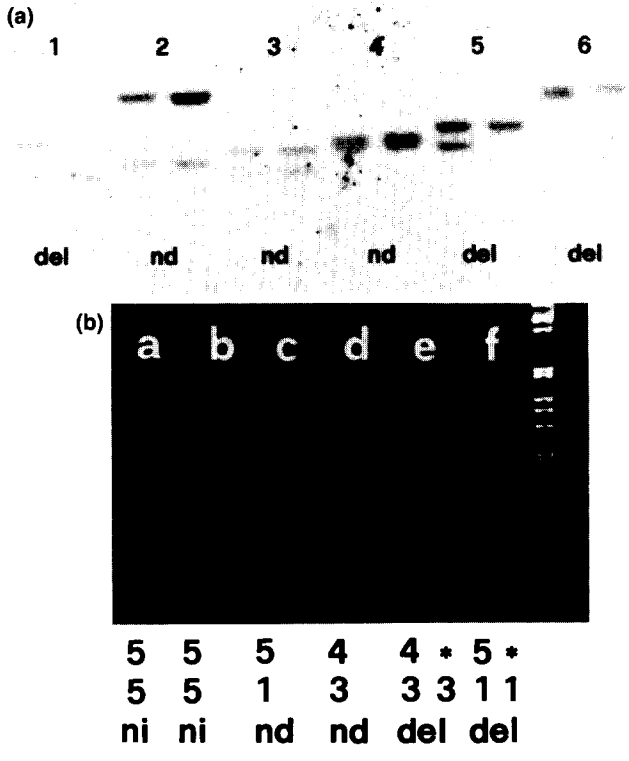

Figure 4. Allelic loss analysis at 17p13 (*D17S30*) in NSCLC. (a) Southern blot analysis of six normal lung tissue-NSCLC tissue pairs with the pYNZ22 RFLP probe and a HinfI Southern blot (del, heterozygous and allelic deletion; nd, heterozygous but no allelic deletion). (b) AFLP-PCR analysis of six normal lung tissue-NSCLC tissue pairs using pYNZ22-specific PCR primers. Allele sizes and allele status are given below each tissue pair (ni, homozygous thus not informative; nd, heterozygous but no allelic deletion; del, heterozygous and allelic deletion).

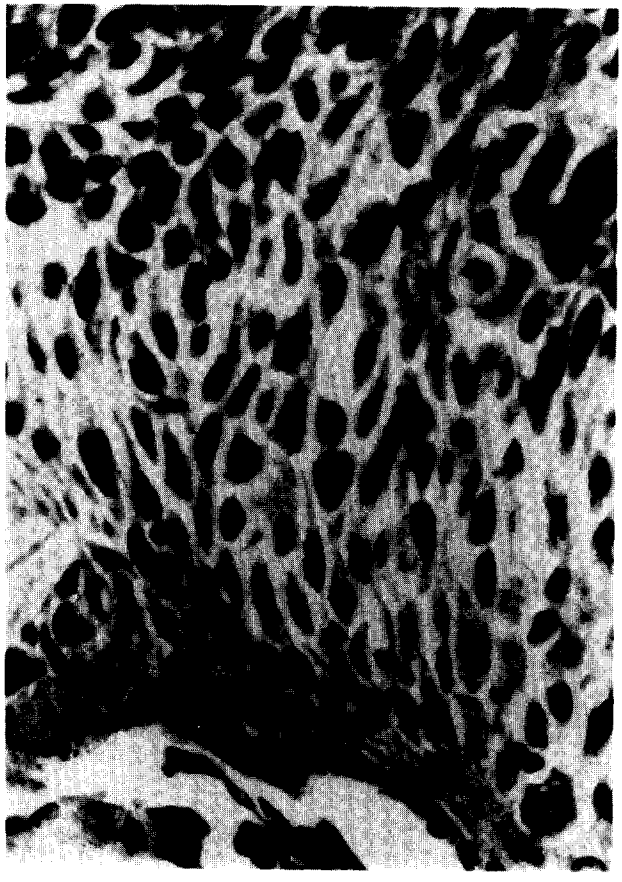

Figure 5. Immunohistochemical analysis of p53 expression in NSCLC. Positive staining of cell nuclei for p53 protein accumulation with a polyclonal antip53 antiserum (OPA 28/2). The staining pattern is exclusively nuclear with no evidence of cytoplasmic p53 protein accumulation (× 1000 magnification).

DISCUSSION

Direct sequence analysis of the *TP*53 gene in 24 primary, resected non-small cell lung carcinoma samples revealed mutations in 9 of 24 cases (38%). The distribution of mutations in our study differs with respect to tumour type from that reported for total lung cancers (Table 1; [3–5]). This is due, in part, to the composition of our study group (13 SQC, 8 LLC and 3 ADC) and the fact that the number of cases examined is too small to permit definitive conclusions on the basis of statistical analysis. The majority of *TP*53 point mutations occur within the five HSRs comprising exons 5 and 8 [5]. The 3 bp (agc) deletion in tumour 16 deletes one amino acid from the predicted protein sequence ($Cag^{gln}.cAC^{his} > CAC^{his}$). The effect of this single amino acid deletion on the activity of *TP*53 is uncertain, but the deletion occurs three amino acids "upstream" from the conserved domain III and could alter or ablate the DNA binding ability of *TP*53. In addition, three NSCLCs displayed multiple point mutations which were detected and confirmed by DNA sequencing and SSCP-PCR analysis. Two NSCLCs displayed a double point mutation on one of the *TP*53 alleles while a third

NSCLC displayed a single point mutation on one allele and a triple point mutation on the remaining allele. The mechanism of allele bias for the multiple mutations observed in these three NSCLCs is unclear, but has been observed previously and may indicate a DNA repair bias for the expressed versus the non-expressed allele [34, 35]. If the non-expressed allele is not repaired efficiently, the opportunity for accumulation of more than one mutation would be increased.

The mutations residing on this allele are revealed only by secondary mutation or deletion of the wild-type allele resulting in p53 accumulation.

Ten NSCLC samples with wild-type (wt) *TP53* sequence displayed allelic loss and/or p53 protein expression. These cases could harbour mutations of the *TP53* gene outside the region analysed in this study but such mutations do not occur frequently in NSCLC [5]. In the context of a wt *TP53* gene, the *D17S30* allelic loss might indicate that another gene locus, in addition to *TP53*, is involved in tumour progression [36, 37] or that loss of one *TP53* allele is sufficient for tumour progression [38]. Alternate mecahnisms for wt *TP53* accumulation have also been proposed which invoke the inactivation of the p53 degradation pathway, stabilisation of wt *TP53* through complex formation with viral or other cellular protein(s) or the altered expression of *TP53* by cellular transcription factors [24]. In this respect, our data support the contention that alterations of wt *TP53* activity contribute to tumour development by permitting the survival of potentially malignant cell clones through an improper response to DNA damage or the induction of apoptosis in these cells. This point is accentuated by comparison of *TP53* mutations in cell lines and primary tumours. Nearly all cell lines established from primary tumours display homozygous *TP53* mutations indicating a selective growth advantage for such cells [39, 40]. In this regard, the cellular heterogeneity of primary NSCLCs may result in an underestimate of the number of *TP53* point mutations due to the presence of non-cancerous cells within the tumour sample. Studies in transgenic mice that overexpress a mutant p53 protein further implicate *TP53* mutation in the molecular processes underlying lung tumour development since such transgenic mice exhibit a high incidence of lung tumours [41].

Of the NSCLC cases, 59% exhibited *D17S30* allelic loss, an incidence similar to previous NSCLC studies (Table 1 [42, 43]). All of the NSCLCs with single point mutations were also deleted at *D17S30*. In the cases where *TP53* mutation was not accompanied by allelic loss, the point mutations may give rise to a dominant negative *TP53* gene which does not necessarily require the deletion of the remaining allele for activity. In the cases where allelic loss was not accompanied by *TP53* mutation, it is possible that the loss of other tumour suppressor genes at this locus permit the accumulation of p53 and thereby aid in the development of the NSCLC. In fact, allelic loss at *TP53* does not occur as often as allelic loss at *D17S30* in *TP53* mutant tumours suggesting that deletion of the remaining *TP53* allele is not obligatory and that loss of a gene(s) at *D17S30* may be more meaningful for the expression of mutant *TP53* in these tumours [18, 44].

Of the NSCLC cases, 71% displayed nuclear accumulation of the p53 oncoprotein (Table 1). Of the NSCLC cases with mutant *TP53*, 100% (9/9) were immunopositive for mutant p53 protein in the nucleus, where its expression presumably interferes with the function of wt *TP53* [45–47]. Similar to previous reports, 53% (8/15) of the NSCLC cases displaying wt *TP53* sequence were also immunopositive, implying the existence of alternate genetic mechanisms leading to p53 accumulation in NSCLC [20, 26, 48]. Although biased intracellular distribution of mutant p53 protein has been reported [39, 49–51], none of our NSCLC samples showed a cytoplasmic staining pattern.

Previous studies examining the association between *TP53* alterations and clinicopathological parameters in NSCLC have yielded conflicting results and have failed to find significant correlations with respect to known prognostic factors [52]. In our study, no significant correlations were observed between individual genetic alterations or between these alterations and the relevant tumour parameters. However, the results of our study indicate that genetic alterations of the *TP53* gene occur frequently in primary, non-pretreated NSCLC. In light of our observations of single and multiple *TP53* point mutations in NSCLC and in analogy to the findings for squamous cell carcinoma of the lung [25] and the skin [34], a reduced apoptotic potential of bronchial epithelial cells heterozygous for *TP53* point mutations could support the accumulation of additional mutations in the *TP53* gene, and in other growth regulatory genes, and thereby contribute to tumour development in NSCLC.

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Acknowledgement—This study was supported by SNF grant 31-30098.90 from the Swiss National Research Foundation.