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Original Paper

Genome Wide Search for Genetic Damage in p53 Transgenic Mouse Lung Tumours Reveals Consistent Loss of Chromosome 4

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The tumour which develops most frequently in mice carrying a *p53* Val^{135} transgene is adenocarcinoma of the lung. We established 10 cell lines from these tumours and investigated their karyotypes by detailed cytogenetic analysis using a complete set of mouse chromosome-specific paints. Consistent loss of chromosome 4 material was noted in 9 out of 10 cell lines: this loss was detected in tetraploid but not diploid cells of the same cell line, suggesting that mouse chromosome 4 plays a critical role in the progression of lung adenocarcinomas. Other frequently observed chromosome aberrations involved chromosomes 7, 5 and 8. Atypical bronchial epithelium was observed together with lung tumours and in tumour-free, apparently normal lungs indicating that mouse lung tumours induced due to the presence of a mutant *p53* transgene may develop via pre-invasive lesions and thus may be effective models for the study of lung tumour progression. © **1997 Elsevier Science Ltd.**

Key words: mouse chromosome 4, lung adenocarcinoma, p53 transgenic mice, chromosome paint, mouse model

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INTRODUCTION

MOST PATIENTS with non-small cell lung cancer (NSCLC) have advanced stage disease at presentation. The disseminated nature of NSCLC makes it almost incurable in spite of vigorous combined modality treatment. Adenocarcinoma is the histological subtype that is most frequently metastatic, least associated with cigarette smoking and increasing in incidence relative to other subtypes of NSCLC. It is clear that current therapeutic strategies are inadequate and future approaches require better understanding of the pathogenesis of this disease.

Multiple genetic alterations are involved in the development of lung adenocarcinoma. Cytogenetic studies of adenocarcinomas have disclosed complex chromosome abnormalities, predominantly losses of chromosome 3p, 9p, 13q, 17p and Y [1], while gain of chromosome 7 has been observed frequently [1]. Allele loss studies have confirmed the observation of these losses, and additionally loss of heterozygosity on chromosomes 2q, 8q, 11q and 17q have also been detected in adenocarcinoms [2–4]. Some of the studies, which compared differences in allelotype between adenocarcinoma and squamous cell carcinoma of the lung, have identified common genetic features [2, 3], but also differences between these two histological subtypes [2–4]. In general, the frequency of a common genetic abnormality, 3p loss for example, is lower in adenocarcinomas than in other histological subtypes of lung tumours [3]. This may be a reflection of the difficulties of allele loss studies using adenocarcinomas since they are frequently admixed with a high proportion of normal cells which may obscure the tumour genotype. None of the studies referred to above, designed to investigate allelotypes of adenocarcinomas, employed microdissection, a procedure which greatly decreases stromal contamination. Therefore, it is possible that the proportion of a specific allele loss such as 3p was inevitably underestimated.

Mutation in the genes for p53 (5) and K-ras [5, 6] have been detected in human lung adenocarcinomas. *K-RAS* mutation may be unique to this histological subtype and is certainly associated with a poor prognosis [6]. Involvement of

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9p, first detected on cytogenetic analysis, has now been shown to be due to inactivation of the *CDKN2* gene at 9p21-22, either by homozygous loss or mutation [7, 8].

Mouse lung tumours share many features of human lung adenocarcinoma including morphology, anatomical origin, biochemical phenotypes [9] and some of the genetic characteristics. Although *p53* mutations are rare in mouse lung tumours [10, 11], mutations in the K-ras gene have been frequently detected [12, 13]. Mouse lung tumours have been shown to have loss of chromosome 4 in several independent studies [14–17]. Deletion mapping analyses using polymorphic markers on this chromosome have revealed that some tumours have homozygous loss of approximately 2Mb in a region around *D4Mit77* syntenic to the 9p21–22 region in human lung cancer [15]. If the mouse *Cdkn2* gene is shown to be involved in lung tumours, as these observations imply, then mouse adenocarcinomas have several important somatic genetic changes in common with their human counterparts.

Mouse lung tumours, which occur spontaneously only in mice bred for susceptibility [18], can be induced by a variety of carcinogens and have been shown to develop in mice carrying mutant *H-ras* [19] and mutant *p53* transgenes [20]. Whereas, all chemically treated mice and those carrying the *H-ras* transgenes develop lung tumours, this is not true of mice with the *p53* mutant transgene as, in these, the tumours are only present after 9 months of age and not in all mice carrying the transgene [20]. Further accumulation of genetic and/or epigenetic alterations must be required. Because enhanced proliferative potential in culture of various epithelial cells from *p53* deficient mice has been observed [21], as well as the scarcity of *p53* mutations detected in mouse lung tumours, continuous growth of epithelial cells in *vitro* by mutant *p53* transgenes could easily give rise to reproducible permanent cell lines used for the studies of genetic alterations in mouse lung tumours. Thus, the development of lung tumours in mice carrying the *p53* transgene may be a particularly useful mouse model of the multistep nature of the equivalent human disease.

The genetic changes underlying human epithelial tumour development have been analysed using both molecular and cytogenetic approaches, but only the former method has been used to any extent in the analysis of mouse lung tumours due to the difficulty of distinguishing mouse chromosomes. To gain more insight into the chromosome aberrations arising in the process of mouse lung adenocarcinoma development driven by the presence of the *p53* transgene (Ala 193 → Val), we have established 10 lung adenocarcinoma cell lines from mice carrying the *p53* transgene. A complete set of mouse chromosome-specific paints was used for detailed cytogenetic analysis of these cell lines. Although clonal heterogeneity was evident, several recurrent chromosome aberrations have been identified. Distinct non-random loss of chromosome 4 was noteworthy in these cell lines, suggesting that mouse chromosome 4 may play a crucial role in lung adenocarcinoma development induced not only by chemical induction but also by the mutant *p53* transgene.

MATERIALS AND METHODS

Mice

The *p53* transgenic mouse strain (CD-1, line p53-2, Val 193 mutation) and the method of confirming *p53* genotypes of each mouse have been described [20]. All mice were monitored for tumour development up to 18 months. When a mouse displayed an obvious tumour or looked unhealthy, necropsy was performed.

Cell culture

Each lung tumour specimen was resected and divided to obtain a sample for histopathological diagnosis and a sample for cell line establishment. Resected tumour specimens obtained immediately after necropsy were rinsed twice with RPMI-1640 supplemented with penicillin G (1000 U/ml) and streptomycin (1000 mg/l) and finely minced with surgical scalpels under aseptic conditions. Tumour fragments were then seeded in to a 25 cm^2 plastic flask (Falcon Plastics, Cowley, Oxford, U.K.) containing 0.5 ml Dulbecco's modified Eagle's medium (Sigma, Poole, Dorset, U.K.) supplemented with 10% fetal bovine serum, 2 mmol/l glutamine, penicillin G (100 U/ml) and streptomycin (100 mg/l). The flask was inverted into the up-side-down position to enhance attachment and 4.5 ml medium was added. Cells were incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air in the inverted position. After 2 h incubation, the flask was gently turned to its normal position allowing the medium to cover tumour fragments without detaching them. After the first week, cultures were given a two-third medium change at least twice a week. Subculturing was performed with EDTA–trypsin, and cell suspensions were diluted only 2-fold for the first few subcultures. Thereafter, culture split ratios of 1:5–1:10 were employed. All cultures were routinely tested for mycoplasma contamination.

Chromosome preparation

The third to the sixth passage of lung tumour cultures were employed in the cytogenetic study. In order to obtain long chromosomes suitable for interpreting complex aberrations, 5'-bromodeoxyuridine (BrdU, 200 μg/ml) was added to cultures and incubated for 16 h. Cultures were washed with phosphate-buffered saline (PBS), medium containing 10 μg/ml BrdU was added and the cell lines were incubated for a further 4 h. Metaphase cells were arrested by exposure to colcemid (0.01 μg/ml) 1 h before harvest. Cells were detached from the surface of flasks using EDTA–trypsin, treated with 0.075 M KCL for 10 min at 37°C, and fixed with 3:1 methanol–acetic acid. After several washes in this solution, cells were stored at −20°C until required. Slides were prepared, air dried and aged at 65°C for 3 h.

In situ *hybridisation*

Each mouse chromosome paint, except chromosome 10 and 13 which were only available as a mixture, was hybridised to ribonuclease A pre-treated slides using the procedure described previously [22], except that BrdU incorporation in chromosome preparation was used to ensure early replicating banding patterns (RB banding). Rearrangements involving two or more chromosomes were confirmed by a previously described method for dual-colour detection [23].

Visualisation

Fluorescent signals were detected using a Biorad MRC 600 confocal laser scanning microscope and images were collected and stored on optical discs, then transferred to the

Table 1. Histopathologic features and karyotypes of p53 transgenic lung adenocarcinoma cell lines

Mouse No.	Age (months)	*p53* transgene status	Histological subtype*	Cytological differentiation	Karyotypes
p53-613	18	+	Adenocarcinoma (F)	Poorly differentiated	72-73 < 4n>, XXYY, – Y, – 4, – 4, – 5, – 7, – 9, – 16, – 16
p53-622	18	+	Adenocarcinoma (F)	Poorly differentiated	73-77 < 4n>,XXXX, – X,del(1), – 2, – 4, – 4, + 5, + 5,del(7),der(7)t (7;?), – 8, – 10 or –13, – 11, – 14,del(14), – 16,del(16), – 17, + 18
p53-659	18	+	Adenocarcinoma (C)	Poorly differentiated	39-42 < 2n>, XY, + Rb(X;4), – Y,Rb(Y;4), + 2, + Rb(4;7),del(14)[68.5%]/ 78-84 < 4n>, idem × 2, – Rb(X;4) × 2, – Rb(Y;4), – 2,i(4),Rb(4;16)[31.5%]
p53-675	18	+	Adenocarcinoma (F)	Poorly differentiated	40-41 < 2n>,XY, + 17[24.7%]/77-79 < 4n>,XXYY, – 4, – 14, – 16, + 19[75.3%]
p53-685	16	+	Adenocarcinoma (A)	Well differentiated	116-120 < 6n>,XXXYYY, – X, – Y, –Y, + 1, – 2, – 4, – 4, + 5, + del(5), + 6, + 6, + 6, + del(6) × 2, + 7, –8, – 9, – 11, – 11, – 12, – 12, – 14, + 18, + 19
p53-738	18	+	Adenocarcinoma (F)	Well differentiated	76-78 < 4n>,XXYY, – Y, – 4, – 4, + 8, – 14
p53-800	16	+	Adenocarcinoma (A)	Well differentiated	75-79 < 4n>,XXYY, – Y,del(Y),del(3),del(4),der(5)t(5;11), – 7,del(7), + 8,der(11)t(11;5), – 12, – 14, – 16,der(16)t(16,18), + 19,+ 19
p53-823	7	+	Adenocarcinoma (F)	Poorly differentiated	79-83 < 4n>,XXXX, – X, – 2, – 4, + 6, + 6, + 6,der(8)t(8;14),der(?)t(? ;7),
p53-893	14	+	Adenocarcinoma (F)	Poorly differentiated	56-60 < 3n>,X,der(X)t(X;7) × 2, + 3, – 4, – 5, +der(7)t(7;X), – 9, – 11, – 10 or –13, + 14, + 18
p53-899	14	+	Adenocarcinoma (F)	Poorly differentiated	42-43 < 2n>,XY, + del(5), + 6, + 8[36.6%]/84-85 < 4n>,idem × 2, – 4, +5[63.4%]

*The histological subtype refers to the classification of small adenocarcinomas of the lung listed by Noguchi and associates [29]. A, localised bronchioloalveolar carcinoma (LBAC); C, LBAC with *foci* of active fibroblastic proliferation; F, papillary adenocarcinoma with compressive and destructive growth.

Macintosh Quadra 950, where the composite image was generated using SmartCapture Software (Digital Scientific, Cambridge). For dual-colour detection, fluorescent signals were captured using a Digital Scientific Fluorescent *in situ* Workstation comprising a Zeiss Axioskop microscope, a cooled CCD camera (Photometrics, Tucson, Arizona, U.S.A.) with computer-operated filter wheel and Macintosh Quadra 950.

Evaluation of the chromosome painting results

Evaluation of the preparations was performed by examining at least 25 metaphase spreads for each chromosome paint. Clonal abnormalities were defined and described according to ISCN-1195 [24].

RESULTS

Tumour incidence in mice positive for the p53 transgene

In this study, all suspected tumours were collected, but only those originating in the lung were assessed by microscopy of haematoxylin and eosin stained sections. Histopathological features are described in Table 1. We identified 23 lung tumours from a total of 172 *p53* positive transgenic mice: equivalent to 13.4%. In contrast, tumour incidence in *p53* negative littermates (2 in 111) was very low, i.e. less than 2.0%. Because of our interest in the early stage of human lung cancer development [25, 26], we examined macroscopically normal lungs from *p53* positive mice. Of the 12 mice examined, half showed some evidence of atypical bronchial epthelial morphology: an example of this is shown in Figure 1. Unexpectedly, 4 of these 12 apparently normal lungs showed evidence of tumours not visible on macroscopic examination, three non-Hodgkin lymphomas and one sarcoma (spindle cell).

Karyotypes of p53 transgenic lung adenocarcinoma cell lines

Of the total of 25 mouse lung tumours obtained, 17 were established as cell lines, all from *p53* positive mice. The karyotypes of 10 of these cell lines analysed by chromosome painting are presented in Table 1. All the cell lines exhibited complex karyotypes, mainly numerical and some structural chromosome abnormalities. However, some cell lines had a relatively simple chromosome complement, for example, p53-738. Aneuploidy was observed in all cases with modal chromosome numbers clustering in the near-tetraploid range. Of particular interest were the three cell lines which were a mixture of near-diploid and near-tetraploid clones (p53-899, p53-659, p53-675). One of these, p53-675, may be a mixture of two independently arising clones as tetraploidation of the near-diploid clones would have resulted in six copies of chromosome 17, but only four were observed. (Table 1). However, the near-tetraploid clones of both p53-899 and p53-659 have many chromosomal abnormalities in common with their near-diploid clones and are therefore likely to have developed from them.

Frequencies of the numerical and structural chromosome aberrations in each cell lines are listed in Table 2. All chromosomes contributed to numercial abnormalities except chromosome 15. Chromosome losses were much more common than gains. Abnormalities involving chromosome 4 were seen in all 10 cell lines analysed. In 8 lines, there was whole homologue loss; in one line, p53-800, there was a deletion—del(4)(C3) see Figure 2 and in the remaining line, there was an isochromosome 4 and Robertsonian trans-

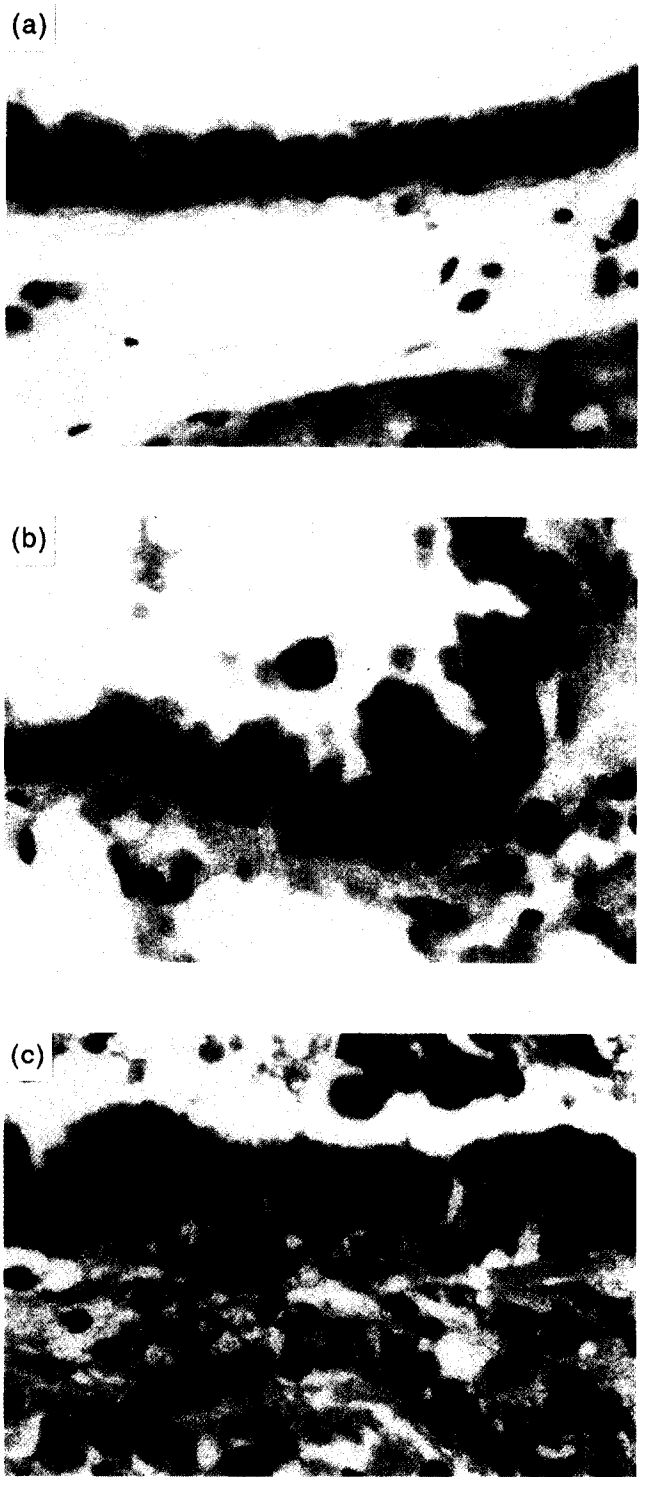

Figure 1. Comparison of normal and atypical bronchial epithelia in *p53* transgenic mice. (a) Normal bronchial epithelium from a non-tumour bearing mouse, p53-1115 (×400). Ciliated columnar cells, with relatively small nuclei. (b) Mild atypia from a macroscopically non-tumour bearing mouse, p53-665 (×400). Focal loss of nuclear polarity with mild cytological atypia and nuclear irregularity. (c) Dysplasia in bronchial epithelium from a macroscopically non-tumour bearing mouse, p53-738 (×400). Marked loss of nuclear polarity, high nuclear:cytoplasmic ratio and nuclear clearing and pleomorphism.

Table 2. Frequency of numerical and structural abnormalities of adenocarcinoma cell lines

Chromosome	p53-613	p53-622	p53-685	p53-738	p53-800	p53-823	p53-893	p53-899 (2N)	p53-899 (4N)	p53-659 (2N)	p53-659 (4N)	p53-675 (2N)	p53-675 (4N)
1		del(1) 24%	+1(56%)										
2		−2(28%)	−2(60%)			−2(33.2%)				+2(56%)	+2(69%)		
3					del(3)12%		+3(32%)						
4	−4 × 2 (87.7%)	−4 × 2 (100%)	−4 × 2 (100%)	−4 × 2 (80%)	del(4)36%	−4(55.5%)	−4(100%)		−4(79%)		i(4)40%		−4(33.3%)
5	−5(73%)	+5 × 2 (88%)	+5(72%) +del(5)8%		der(5) t(5;11)44%		−5(92%)	+del(5) 62.5%	+5(29%) +del(5) × 2 53%				
6			+6 × 3 (88%) +del(6) × 2 (44%)			+6 × 2 (41.0%) +6 × 3 (52.6%)		+6(100%)	+6 × 2 (92%)				
7	−7(93.5%)	der(7) t(7;?)32% del(7)(12%)	+7(88%)		−7(12%) del(7)12%	der(?)t(?;7) 12.2%	+der(7) t(7;X)75%			+Rb(4;7) 2%	+Rb(4;7) × 2(14%)		
8		−8(56%)	−8(92%)	+8(12%)	+8(16%)	der(8)t(8;14) 39.8%		+8(93%)	+8 × 2 (100%)				
9	−9(94.3%)		−9(48%)				−9(40%)						
10 or 13		−10 or −13(76%)					−10 or −13(72%)						
11		−11(88%)	−11 × 2 (100%)		der(11) t(11;5)64%		−11(68%)						
12			−12 × 2 (100%)		−12(28%)								
14		−14(52%) del(14)28%	−14(12%)	−14(40%)	−14(76%)		+14(84%)			del(14) 88%	del(14) × 2(60%)		−14(52.6%)
15													
16	−16 × 2 (95.3%)	−16(88%) del(16)12%			−16(86%) der(16)t (16;18)28%						Rb(4;16) 9%		−16(33.3%)
17		−17(100%)										+17(100%)	
18		+18(100%)	+18(32%)				+18(32%)						
19			+19(8%)		+19(32%) +19 × 2 (68%)								+19(42.8%)
X		X(28%)	X(100%)			−X(35.8%)	der(X)t(X;7) × 2(92%)			Rb(X;4)6%			
Y	−Y(18.1%)		−Y(100%)	−Y(20%)	del(Y)12%					Rb(Y;4)28%	Rb(Y;4) 9%		
			−Y × 2(24%)		−Y(16%)					−Y(69%)	−Y × 2 (63%)		

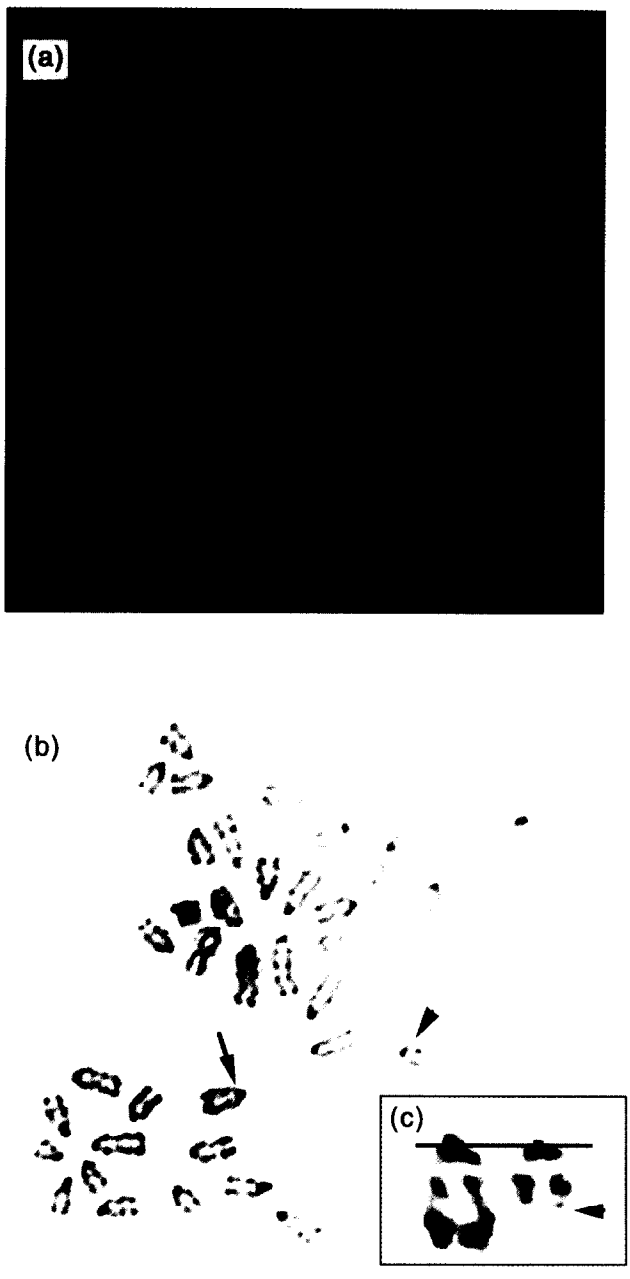

Figure 2. Deletion in chromosome 4 in p53-800. (a) Hybridisation of chromosome 4 paint to a metaphase spread of p53-800. (b) The same spread with an early replicating banding pattern (RB banding, see Materials and Methods) which illustrates the normal chromosome 4 (arrow) deletion del(4)(C3) (arrowhead). (c) Magnification of the normal and deleted chromosome 4 with an arrowhead at the breakpoint.

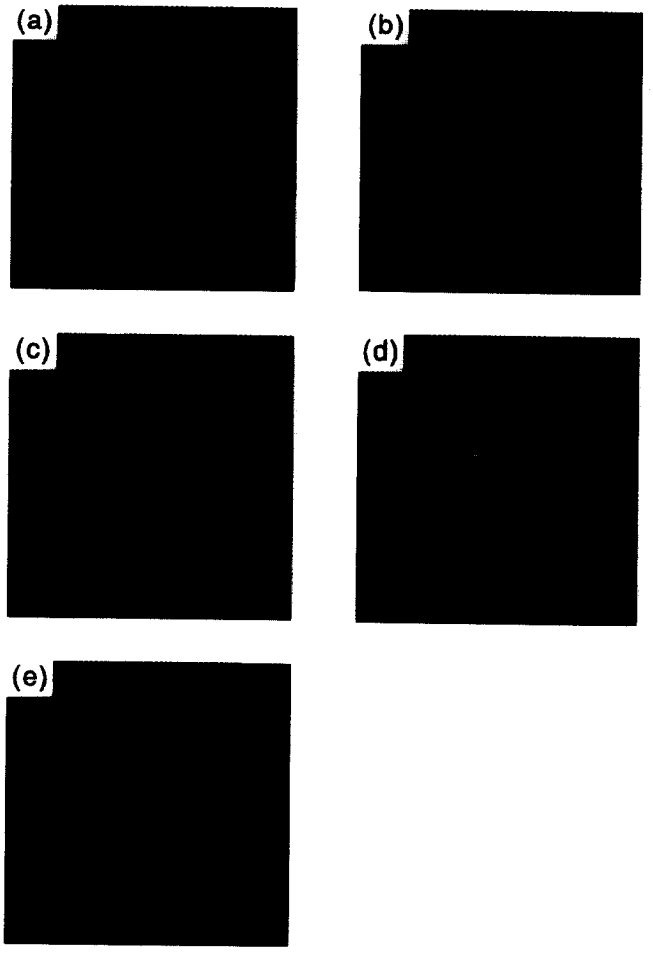

Figure 3. Robertsonian translocations of chromosome 4 in p53-659 metaphase spread. In each panel, the arrowhead indicate the derivative chromosomes. (a) Identification of an isochromosome 4: this occurred in 40% of the metaphases. (b) Identification of a Robertsonian translocation Rb(4;16): this occurred in 4% of the metaphase spreads (chromosome 4 is green). (c) Identification of a Robertsonian translocation Rb(4;7): this occurred in 4% of the metaphase spreads (chromosome 4 is green). (d) Identification of a Robertsonian translocation RB(X;4): this occurred in 4% of the metaphase spreads (chromosome 4 is green). (e) Identification of a Robertsonian translocation Rb(Y;4): this occurred in 24% of the metaphase spreads (chromosome 4 is red).

locations (Figure 3). Three of the 10 cell lines had chromosome 4 loss in all the metaphases examined: these cell lines had no near-diploid population. Of the three cell lines which were mosaics of near-diploid and near-tetraploid cells (p53-659, p53-675 and p53-899), two had chromosome 4 loss which occurred only in the near-tetraploid subpopulation. This is illustrated for the cell line p53-899 in Figure 4, which shows that the near-diploid population had two chromosomes (per homologue) and the near-tetraploid, 4 chromosomes (per homologue), as demonstrated using chromosome paints. Chromosome 1 is shown as an example (Figure 4(a) and (c)). The near-diploid population of p53-899 has two copies of chromosome 4. However, the near-tetraploid cells had only three copies of chromosome 4 in most of the metaphases examined indicating loss of chromosome 4 during tetraploidation (Figure 4 (b) and (d)). Other chromosome losses, observed in three or more cell lines, involved, 2, 9, 11, 14, 16, X and Y. Recurrent gains of chromosome 5, 6, 8, 18 and 19 were prevalent.

Unbalanced, clonal rearrangements (deletions and derivative chromosomes) seem to frequently involve chromosome 7. An isochromosome 4 was identified in a cell line (p53-659) in which Robertsonian translocations of chromosome 4 and chromosome 7, 16, X & Y were identified (Figure 3).

DISCUSSION

Lung tumours are uncommon in mice except in those strains bred for tumour susceptibility. Unlike human lung

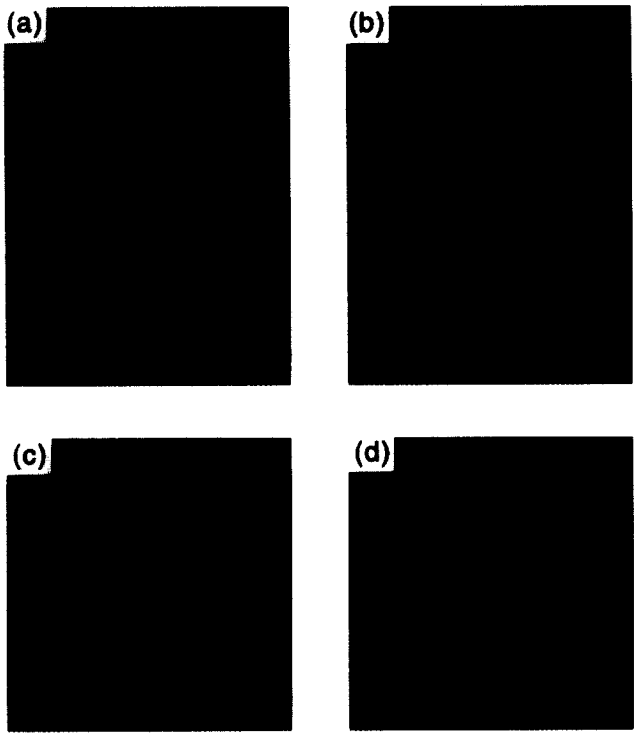

Figure 4. Demonstration of chromosome 4 loss in the near-tetraploid subpopulation of p53-899. (a) and (b) are metaphase spreads of the near-diploid subpopulation of p53-899 hybridised with paints for chromosomes 1 and 4, respectively. (c) and (d) are metaphase spreads of the near-tetraploid subpopulation of p53-899 hybridised with paints for chromosomes 1 and 4, respectively.

tumours, neither spontaneous nor chemically-induced mouse lung tumours show frequent *p53* mutation [10, 11], although they do carry other genetic changes in common with human adenocarcinomas, most notably, K-ras mutations and deletions in chromosome 4 equivalent to those seen in 9p in human tumours encompassing the *MTS1/CDKN2a* and *MTS2/CDKN2b* loci [15, 16]. Thus, it was particularly noteworthy that mice carrying a *p53* mutant transgene developed lung tumours as the most frequent tumour type [20]. We confirmed that lung tumours occurred frequently in our *p53* transgenic mice colony. Our observation of macroscopically undetectable tumours and bronchial epithelial atypia in apparently normal lung suggests that the true incidence may have been underestimated.

The study of genetic changes in human lung tumours has benefited from a combination of cytogenetic and molecular genetic approaches, the latter usually refining and confirming observations made by the former. However, owing to the difficulty of distinguishing mouse chromosomes, karyotype analysis has had a very minor role in defining genetic changes in lung (and other) tumours in the mouse. Notwithstanding this problem, the number of reported cytogenetic analyses of lung tumours is still surprisingly few. DiPaolo has described numerical chromosome changes in mouse lung tumours [27], but this report pre-dated the development of the chromosome banding technique. In fact, to date, only two karyotypes of mouse lung tumours have been published [28]. In this study, we have taken advantage of the ease with which lung tumours carrying a *p53* transgene can be established in tissue culture to describe 10 complete karyotypes using chromosome-specific paints. Not only is this an extremely repaid method of cytogenetic analysis, but in our work on human lung tumours, when we have compared karyotypes produce by G-banding and chromosome painting, the latter has revealed chromosomal rearrangements not detected by the former. Thus, we believe that this method of karyotype analysis is both more accurate and more rapid for providing an overall picture of a tumour karyotype, although by its nature, some abnormalities, for example, chromosomal inversions, will be missed.

In this study, we showed that loss of chromosome 4, previously inferred by molecular genetic analysis in carcinogen-induced lung tumours, was also important in lung tumours arising in mice due to the presence of the *p53* transgene. This similarity implies that the loss of chromosome 4 may not be a direct consequence of the presence of the mutant *p53* transgene but rather an absolute requirement for mouse lung tumour development irrespective of initiation. The effects of the transgene may be directed toward other chromosomes, perhaps those frequently altered in these mouse lung tumours, for exmaple, chromosomes 5, 7 and 8. Alternatively, the mutant *p53* transgene may cause genetic instability, resulting in random genetic loss which favours lung tumour development when the loss involved chromosome 4. In our study, 9 out of 10 cell lines showed loss of chromosome 4 material. A lower proportion was noted in the study of Herzog and associates [17], although this may relate to their use of biopsy material and LOH analysis as opposed to direct observation of cell lines. The study described by Herzog and associates concluded from the extensive loss of alleles on chromosome 4 that the mechanism of allele loss was likely to be non-disjunction. Our direct observation of tumour chromosomes confirms this as the mechanism of loss of chromosome 4 material in lung tumours arising in mice carrying the *p53* transgene. In the study of Herzog and associates, LOH on chromosome 4 was compared in adenocarcinomas and adenomas. Apart from 1 of 38 adenomas, only the adenocarcinomas showed chromosome 4 loss, implying tht inactivation of gene(s) on this chromosome are implicated in the transition to malignancy. In our study, there was also evidence that loss of chromosome 4 was involved in lung tumour progression. We described two cell lines which were a mixture of near-diploid and near-tetraploid cells. Only the near-tetraploid cells demonstrate chromosome 4 loss and the more complex karyotypes are presumed to be the more advanced.

Although complete loss of chromosome 4 material is often detected in mouse lung tumours, a few chemically-induced tumours carry deletions which allow better definition of the location of potential tumour suppressor genes involved in mouse lung cancer development [14–17]. Three tumours with homozygous deletions around *D4Mit77* have been characterised by Herzog and associates. This region of approximately 2cM is syntenic with the human 9p21 region where CDKN2a and *CDKN2b* are located [15]. This same group has also described the loss of a more distal

A.C. Heppell-Parton, E. Nacheva, N. Cartner, J. Bergh, D. Ogilvie and P.H. Rabbitts. Elucidation of the mechanism of homozygous deletion of 3p12-13 in the U2020 cell line reveals the unexpected involvement of other chromosomes, manuscript in preparation

region on chromosome 4 around 3cM between *D4Mit251* and *D4Mit170*, which corresponds to 1p36 in the human karyotype [17]. The authors suggest that, in view of the frequent observation of loss of the whole chromosome 4, then both regions probably require inactivation for malignancy. The cell line, p53-800, described above, has a deletion rather than whole homologue loss but this encompasses both of the regions of loss described by Herzog and associates.

Mouse lung tumours have not always proved to be a good model of human lung tumours, particularly with respect to the effect of chemotherapeutic agents [30]. However, when the genetic aetiology of the disease is being considered, mouse lung tumours appear to show features in common with human lung tumours. In this study, we have shown that lung tumours induced in mice due to the presence of the *p53* transgene may also have features in common with their human counterparts. Because the lung tumours which develop in the *p53* transgenic mice present late in their life history are not inevitable (not all mice carrying the *p53* transgene develop tumours—in contrast to carcinogen-exposed mice) and show evidence of accompanying/preceeding epithelial atypia, this sytem of tumour induction may be particularly useful as a model system for the study of sequential genetic changes in human lung tumour development.

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