Genetics and pulmonary medicine • 7

Series editors: J Britton, J Hopkin

Somatic mutations in the development of lung cancer

M Roland, R M Rudd

Lung cancer is the commonest malignancy in the Western world and smoking the single greatest risk factor.[1] The growth cycle of lung tumours is such that they have reached an advanced stage in their natural history before an affected individual becomes symptomatic.[2] For this reason advances in treatment have produced only very modest improvements in the prognosis of lung cancer in the past 30 years. Earlier detection might improve results of treatment but, to date, screening by radiography and sputum cytology has proved disappointing.[3]

Studies of the evolution of bowel cancer have shown that cancers develop as a result of serial genetic mutations accumulating over a lengthy time span.[4 5] This has been harder to prove for lung cancer as premalignant lesions are more difficult to identify and biopsy than they are in the bowel. Despite this, certain commonly occurring somatic mutations have now been found to be involved in the evolution of lung cancer. With this knowledge come exciting potential preventative and therapeutic strategies for the future.

Theory of somatic mutation

Genetic mutations in the cells of the body happen naturally at a very slow rate, 10^{-5}–10^{-7} per gene per generation, there being 10^{14} cells in the body.[6] Most such somatic mutations confer no reproductive advantage to the cell involved, and hence no pathological consequences ensue. Even when a mutant cell is able to proliferate, it must then overcome numerous control mechanisms evolved by the body—for example, apoptosis whereby potentially cancerous cells undergo programmed cell death. Six or seven separate genetic mutations are required for a normal cell to evade the control mechanisms and become a cancerous cell. If mutations occur at their normal rate, the probability of one cell sustaining six mutations in its lifespan would be 1 in 10^{22}—that is, negligible.[6] Some mutations, however, increase the probability of subsequent mutations occurring—for example, by enhancing cell proliferation sufficiently to create an expanded target population of cells for the next mutation or increasing the overall mutation rate by affecting the stability of the entire genome.

Knudson extended the somatic mutational hypothesis in 1985 by describing four demographic groups with different expectations of cancer depending upon environmental and hereditary variables.[7] The first group arises solely from spontaneous mutations producing a "background" level of cancer which we cannot reduce without learning how to slow down this natural spontaneous process. This group comprises the 20% of cancers that would remain if all environmentally induced cancers were prevented. The second and largest group comprises those with cancer produced by environmental agents such as chemicals (including the carcinogens contained in cigarette smoke), radiation and viruses which accelerate mutagenesis. In the third group, which may overlap somewhat with the second group, the individual has some genetically determined difference resulting in an increased risk of spontaneous or induced mutations. The classic example of this is xeroderma pigmentosum, an inherited disorder predisposing to skin cancers where patients lack part of an excision and repair mechanism which cuts and replaces inappropriate bonds between thymines formed on exposure to ultraviolet light. In groups two and three the probability of the occurrence of one or more mutations is increased, but the number of mutations required to produce malignant transformation is not reduced. Members of the fourth group, however, inherit an initiating mutation which reduces the number of steps in oncogenesis by one and strongly predisposes the individual to certain specific cancers which are designated as hereditary—for example, colonic cancer associated with polyposis coli, retinoblastoma, and multiple endocrine neoplasia type 2. No hereditary mutation has yet been demonstrated clearly for lung cancer[8] and most cases fall into Knudson's second group.

Not only must there be cumulative somatic mutations before carcinogenesis will occur, but these mutations must occur at certain specific genetic loci which are involved in the regulation of cellular proliferation or repair. It will be necessary to identify these genes and understand their function if we can hope to influence the disease process at a genetic level.

To date, three main groups of genes have been identified which are frequently mutated in cancer—proto-oncogenes, tumour suppressor genes, and mutator or DNA repair genes.

London Chest Hospital, Bonner Road, London E2 9JX, UK
M Roland
R M Rudd

Correspondence to:
Dr R M Rudd.

Proto-oncogenes are normal cellular genes which, when mutated, become oncogenes. Oncogenes code for proteins involved in the control of the cell cycle and, when activated, stimulate cell division inappropriately.[6] Oncogenes are genetically dominant, requiring the allele on only one of the two chromosomes to be altered to have a phenotypic effect. Tumour suppressor genes inhibit cell division in response to DNA damage, delaying the cell cycle until the damage is repaired. Both alleles of a tumour suppressor gene must be inactivated to change the behaviour of the cell. Ultimate loss of tumour suppressor activity requires a mutation to convert from a heterozygous to a homozygous inactivated state and this is described as a "loss of heterozygosity" (LOH).[6] Loss of heterozygosity can be demonstrated by comparing the same chromosomal regions in peripheral blood cells with tumour cells, and where it occurs it is taken to imply the presence of an inactivated tumour suppressor gene in that region. Oncogenes and tumour suppressor genes act through cell cycle controls. The third class, mutator genes, are involved prior to mutation in DNA repair processes. Mutator gene inactivation is recessive, like tumour supressor gene inactivation, so both alleles must mutate to inactivate the normal repair mechanism.

Specific mutations related to lung cancers

Epithelial cancers evolve in a stepwise fashion over time, through a series of morphologically recognisable preneoplastic lesions.[9] Only a small number of these lesions will progress to invasive cancers and some may regress spontaneously or after smoking cessation.[10] These histological changes were postulated to be driven by a sequential series of somatic mutations specific for each tumour type.[11] This was strongly supported initially by molecular genetic studies of colonic tumours carried out in the 1970s made possible because precursor lesions (adenomas) could be readily identified and biopsy specimens taken.[5] As precursor lesions were harder to identify in the lung, identification and sequencing of characteristic somatic mutations has been more difficult.

In the last 15 years the techniques of microdissection and polymerase chain reaction (PCR) have rendered small samples of preneoplastic lung lesions and tumour accessible to genetic studies[12] and fluorescence bronchoscopy renders preneoplastic lesions more amenable to accurate sampling.[13] In microdissection a microcapillary pipette is used to sample cells from a haematoxylin and eosin stained slide without a coverslip under microscopic guidance. The cells are enzymatically digested to free their DNA for PCR analysis. PCR is a method of rapid selective amplification of specific target DNA sequences within a heterogeneous collection of DNA. Two primer sequences 15–30 nucleotides long, complementary to the DNA sequences immediately flanking the target region, initiate the synthesis of the new DNA strands facilitated by DNA precursors and DNA polymerase. In a chain reaction the number of strands of DNA available to act as templates doubles with each cycle. About 10^5 copies of the target sequence will have been generated after about 30 cycles, an amount that can be seen as a discrete band when submitted to agarose gel electrophoresis. Target regions of DNA obtained from suspect lesions can then be compared with equivalent DNA regions obtained from leucocytes in the peripheral blood to detect any loss of heterozygosity.

Several studies have involved taking bronchoscopic biopsy specimens from fixed sites in macroscopically normal airways of asymptomatic current smokers, non-smokers, and ex-smokers looking for dysplasia.[14 15] Others have taken biopsy specimens of tumour, adjacent dysplastic mucosa, and distant mucosal sites and compared the merits of parallel sampling (biopsy specimens taken from all mucosal sites at the same time) with longitudinal sampling (samples taken at different times from the same patient).[16] These studies have supported the concept of a carcinogen exposed field effect whereby much of the respiratory mucosa can be shown to contain somatic mutations which are probably due to exposure to the carcinogens contained in cigarette smoke.[17] A clonal relationship (originating from the same cell) seems now to have been confirmed between adjacent bronchial abnormalities of different grades in the same patient (allele specific mutations) but has not yet been reliably defined between distant lesions.[9 16] This clonal relationship suggests that parallel sampling of different grades of adjacent premalignant and malignant tissue is a legitimate alternative to longitudinal sampling in the study of tumour progression.

Lung cancers have very complex karyotypes so almost any chromosomal abnormality can be demonstrated (table 1). Deletion or loss of heterozygosity on chromosome 3 is the commonest mutation found in both small cell and non-small cell lung cancer and is thought to be one of the earliest mutations seen.[8 9] A number of distinct regions of chromosome 3 are thought to be damaged sequentially as premalignant change progresses. Mutation of the p53 gene at 17p13 is probably the commonest single genetic change seen in all human cancers and is present in the majority of small cell[18] and a smaller percentage of non-small cell lung cancers.[19] Mutation of p53 is thought to occur later in tumorigenesis than chromosome 3 abnormalities. A deletion on 9p is seen in some of the earliest detectable preneoplastic lesions in both small cell and non-small cell cancers.[20 21] The p16 gene at 9p21, originally defined as a tumour suppressor gene locus in leukaemia, seems more commonly involved in the pathogenesis of non-small cell lung cancer than small cell lung cancer, with some other as yet unidentified tumour suppressor gene locus more commonly involved in the latter.

Although no clear hereditary component has been found for lung cancers, deletions on the long arm of chromosome 13 in the region of the retinoblastoma gene have been demonstrated in small cell lung cancer.[22 23] Indeed,

Table 1 Chromosomal abnormalities in lung cancer

Site of mutation	*Name of mutation*	*Frequency*	*Type of mutation*	*Function*
Chromosome 3 (short arm) 3p14.2, 3p21.3, 3p25	Fragile histidine triad (FHIT)	SCLC ~100% NSCLC ~60% (SqCLC ~100%)	Deletion/loss of heterozygosity (LOH)	Tumour suppressor genes; 3p21.3 codes for a mutator gene
Chromosome 8	MYC	SCLC 30–40%	Translocation (8:14, 8:2, 8:22)/amplification	Oncogene transcription factor
Chromosome 9 (short arm) 9p21	p16 (aka CDKN2)	SCLC >80% NSCLC >50%	LOH/small (<500 kb) deletions	Tumour suppressor gene
Chromosome 12 (short arm) 12p	k-Ras	Adeno 30–40%	Point mutations	Oncogene p21. GTPase
Chromosome 13 (long arm) 13q	Retinoblastoma (Rb) gene	SCLC >90% NSCLC less common	Point mutations	Tumour suppressor gene
Chromosome 14–18 translocation	Bcl-2 (B cell lymphoma/ leukaemia 2)	SqCLC 25% Adeno 10%	Chromosomal translocation	Oncogene, inhibits programmed cell death
Chromosome 17 (short arm) 17p13	p53 (aka TP53)	SCLC ~75% NSCLC ~50% (but probably most common single genetic change in all cancers)	Deletion/LOH	Tumour suppressor gene
Chromosome 17 (long arm) 17q	c-erb B-2/neu	NSCLC 30–40%	Chromosomal translocation/ amplification	Oncogene, transcription factor

SCLC = small cell lung cancer; NSCLC = non-small cell lung cancer; SqCLC = squamous cell lung cancer; Adeno = adenosquamous cell lung cancer.

small cell lung cancer resembles retinoblastoma phenotypically and, if successfully treated for retinoblastoma, patients have a 15 fold increase in the risk of developing lung cancer. Important oncogenes associated with lung cancer evolution include k-Ras (k-Ras point mutation tumours are more poorly differentiated than those without),[24 25] Bcl-2 (expression of which seems to confer an improved prognosis on patients with non-small cell lung cancer for reasons which are as yet uncertain),[26 27] NEU oncogene,[28 29] and MYC oncogene (particularly important in small cell lung cancer).[30 31]

Smoking

Smoking remains the single greatest risk factor for lung cancer.[32] There are at least 43 known carcinogens in cigarette smoke. These accelerate somatic mutations, causing a field effect in the respiratory mucosa whereby areas of loss of heterozygosity may be demonstrated throughout the mucosa of the lung, even where no preneoplastic histological abnormality exists. Such preneoplastic histological changes increase in extent and severity with pack years but resolve steadily after smoking cessation. Somatic mutations, however, persist. Wistuba *et al*[14] submitted biopsy specimens from current, former and lifetime non-smokers to PCR analysis. They found no significant difference in the frequency of allelic loss between current and former smokers, with multiple mutations still being demonstrable even 48 years after smoking cessation. No mutations were detected in non-smokers. In a similar study by Mao *et al*[15] overall frequency of LOH was 82% in current smokers compared with 62% in former smokers and less than 10% in lifetime non-smokers. They looked for LOH at three specific loci—Chr 3p14, 9p21 and 17p13—and found it to be significantly less frequent at 3p14 in former smokers, suggesting that mutation at this point is a sensitive marker for current smoking status. The persistence of somatic mutations in former smokers provides a compelling biological explanation for the observation that the risk of former smokers developing lung cancer never fully returns to that of a lifetime non-smoker.[1 33]

Screening

As many small studies accumulate we are learning more of the variety of mutations involved in the pathogenesis of lung cancers. We are also starting to gain an understanding of the order in which some of these mutations occur—for example, allele loss on chromosome 3 appears to occur sequentially since the pattern of allele loss seen in dysplasia is more discrete than that seen in invasive tumours, and allele loss on chromosome 3 precedes damage to the p53 gene.[34] As loss of heterozygosity can be demonstrated even in histologically normal mucosa in smokers and ex-smokers, we clearly need to define the more common sequences of mutations before we can hope to use genetic analysis as a reliable predictor of malignant change. Even then the complex karyotype of lung cancers will still mean that many such sequences remain undefined.

Three very large trials in the USA in the 1970s looked at chest radiography with and without sputum cytology as a means of screening male smokers over 45 years of age for lung cancer and found no improvement in eventual mortality compared with control groups.[35–37] Displaying considerable foresight, the Johns Hopkins team stored their sputum samples and continued to collect data from their study group whilst new tumour markers were sought.[38] In 1994 they described PCR based assays for Ras and p53 gene mutations on sputum samples taken one year before the diagnosis and resection of adenocarcinomas in 15 of their original subjects.[39] Conventional cytological sputum screening had been negative in these patients but Ras or p53 gene mutations were demonstrated in the resected tumours of 10 of the group, with eight having corresponding mutations in their sputum. This study demonstrates the potential of sputum as a readily accessible source of genetic material for future screening projects.

Prognosis

Specific somatic mutations have been shown to be reliable prognostic markers in potentially resectable non-small cell lung cancer.[40 41] For example, p53 mutation has been shown to be a significant independent predictor of death in

early non-small cell lung cancer.[42] Mutation of the Rb gene area halves predicted survival after resection for stages 1 and 2 non-small cell lung cancer.[43] Expression of k-Ras and NEU oncogenes predicts a poor prognosis in resectable non-small cell lung cancer[44 45] whilst five year survival is higher for patients with Bcl-2 positive tumours.[26] Combinations of p53 and k-Ras or c-erb B-2 (NEU) expression have been shown to predict a significantly reduced survival than that expected from the presence of either mutation alone.[42 43]

Implications for treatment

Somatic mutations have potential as a guide to the treatment of lung cancer. The presence of certain mutations which act as poor prognostic markers in potentially resectable non-small cell lung cancer may identify an increased likelihood of benefit from adjuvant chemotherapy. Certain mutations may also indicate a likelihood of resistance to chemotherapy. For example, p53 deletion and Bcl-2 expression render some tumour cells resistant to apoptosis following chemotherapy.[46 47] Expression of the k-Ras oncogene seems to produce a resistance to platinum based compounds and etoposide.[48]

Defining the mutations present in premalignant and malignant lesions may provide us with novel therapeutic strategies involving the elimination of activated oncogene products or the replacement of non-functioning with functioning wild type tumour suppressor genes. Retroviruses are the obvious delivery vehicles for such gene replacements. Some successes have been reported in animal models introducing wild type p53[49] and wild type Rb genes[50] and also an "antisense" (AS) k-Ras gene into tumour cells by this method.[51] For example, one study involved intratracheal inoculation of irradiated mice with large cell lung cancer cells carrying the k-Ras oncogene. Three days later viral supernatant ± AS-k-Ras was inoculated via the same route. At post mortem examination 30 days later 90% of control mice had tumours compared with only 13% of treated mice.[51]

A further potential treatment strategy facilitated by the identification of somatic mutations is the production of modified monoclonal antibodies directed against tumour cells expressing a specifically targeted oncogene. In vivo studies of this method using C-erb B-2 expressing tumour cells have shown potent cytotoxic effects.[52]

Summary

Lung cancers exhibit complex heterogeneous karyotypes and to date sequencing the serial somatic mutations which give rise to malignant change has proved difficult. Cigarette smoke causes a field change in the respiratory mucosa with mutations demonstrable even in histologically normal areas. After smoking cessation many of these mutations seem to persist indefinitely so that the risk of an ex-smoker developing lung cancer never reverts to that of a life-long non-smoker.

Demonstration of specific somatic mutations in biopsy or sputum samples may eventually provide a useful method of screening for lung cancer. Somatic mutations give useful information about prognosis in non-small cell lung cancer and they are the key to exciting future retroviral and monoclonal antibody mediated therapies.

1 Doll R, Peto R. Mortality in relation to smoking: 20 years observation in male British doctors. *BMJ* 1976;2:1525.
2 Geddes DM. The natural history of lung cancer: a review based on rates of tumour growth. *Br J Dis Chest* 1979;**73**:1.
3 George PJM. Delays in the management of lung cancer. *Thorax* 1997;**52**:107–8.
4 Nowell P. The clonal evolution of tumor cell populations. *Science* 1976;**194**:23–8.
5 Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990;**61**:759–67.
6 Strachan T, Read AP. Somatic mutations and cancer. In: Strachan T, Read AP, eds. *Human molecular genetics*.Oxford: Bios Scientific Press, 1996: 457–77.
7 Knudson AG. Hereditary cancer, oncogenes and antioncogenes. *Cancer Res* 1985;**45**:1437–43.
8 Rabbits PH. Genetic changes in the development of lung cancer. *Br Med Bull* 1994;**50**:688–97.
9 Hung J, Kishimoto Y, Sugio K, *et al*. Allele specific chromosome 3p deletions occur at an early stage in lung cancer. *J Am Med Ass* 1995;**273**:558–63.
10 Auerbach O, Hammond EC, Garfinkel L.Changes in bronchial epithelium in relation to cigarette smoking, 1955–60 vs. 1970–77. *N Engl J Med* 1979;**300**:381–5.
11 Foulds L.The natural history of cancer. *J Chronic Dis* 1958; **8**:2–37.
12 Strachan T, Read AP. PCR-based DNA cloning and DNA analyses. In: Strachan T, Read AP, eds. *Human molecular genetics*. Oxford: Bios Scientific Press, 1996: 129–45.
13 Lam S, Macauley C, Hung J, *et al*. Detection of dysplasia and carcinoma in situ with a lung imaging fluorescence endoscope device. *J Cardiovasc Surg* 1993;**105**:1035–40.
14 Wistuba II, Lam S, Behrens C, *et al*. Molecular damage in the bronchial epithelium of current and former smokers. *J Natl Cancer Inst* 1997;**89**:1366–73.
15 Mao L, Lee JS, Kurie JM, *et al*. Clonal genetic alterations in the lungs of current and former smokers. *J Natl Cancer Inst* 1997;**89**:857–62.
16 Chung GTY, Sundaresan V, Haselton P, *et al*. Clonal evolution of lung tumours. *Cancer Res* 1996;**56**:1609–14.
17 Strong MS, Incze J, Vaughan CW. Field cancerization in the aerodigestive tract: its etiology, manifestation and significance. *J Otolaryngol* 1984;**13**:1–6.
18 Takahashi T, Takahashi H, Suzuki H, *et al*.The p53 gene is very frequently mutated in small-cell lung cancer with a distinct nucleotide substitution pattern. *Oncogene* 1990;**6**: 1775–8.
19 Chiba I, Takahashi T, Nau MM, *et al*.Mutations in the p53 gene are frequent in primary, resected non-small cell lung cancer. *Oncogene* 1990;**5**:1603–10.
20 Sidransky D. Importance of chromosome 9p loss in human lung cancer. *J Natl Cancer Inst* 1995;**87**:1201–2.
21 Kishimoto Y, Sugio K, Hung J, *et al*. Allele-specific loss in chromosome 9p loci in preneoplastic lesions accompanying non-small-cell lung cancers. *J Natl Cancer Inst* 1995;**87**: 1224–9.
22 Yokota J, Wada M, Shimosato Y, *et al*. Loss of heterozygosity on chromosomes 3, 13, and 17 in small-cell carcinoma and on chromosome 3 in adenocarcinoma of the lung. *Proc Natl Acad Sci USA* 1987;**84**:9252–6.
23 Yokota J, Akiyama T, Fung Y, *et al*. Altered expression of the retinoblastoma (RB) gene in small-cell carcinoma of the lung. *Oncogene* 1988;**3**:471–5.
24 Rodenhuis, Slebos RJC. Clinical significance of *ras* oncogene activation in human lung cancer. *Cancer Res (Suppl)* 1992;**52**:2665–9s.
25 Mills NE, Fishman CL, Rom WN, *et al*. Increased prevalence of K-ras oncogene mutations in lung adenocarcinoma. *Cancer Res* 1995;**55**:1444–7.
26 Pezzella F, Turley H, Kuzu I, *et al. bcl-2* protein in non-small-cell lung carcinoma. *N Engl J Med* 1993;**329**: 690–4.
27 Walker C, Robertson L, Myskow M, *et al*. Expression of the BCL-2 protein in normal and dysplastic bronchial epithelium and in lung carcinomas. *Br J Cancer* 1995;**72**: 164–9.
28 Schneider PM, Hung MC, Chiocca SM, *et al*. Differential expression of the c-erbB-2 gene in human small cell and non small cell lung cancer. *Cancer Res* 1989;**49**:4968–71.
29 Weiner DB, Nordberg J, Robinson R, *et al*. Expression of the neu gene-encoded protein ($p185^{neu}$) in human non small cell carcinomas of the lung. *Cancer Res* 1990;**50**:421–5.
30 Nau M, Brooks B, Battey J, *et al*. L-myc, a new myc-related gene amplified and expressed in human small cell lung cancer. *Nature* 1985;**318**:69–73.
31 Wong A, Rupper J, Eggleston J, *et al*. Gene amplification of C-myc in small cell carcinoma of the lung. *Science* 1986;**233**:461–5.
32 Fearon ER. The smoking gun and the damage done: genetic alterations in the lungs of smokers. *J Natl Cancer Inst* 1997; **89**:834–6.
33 Halpern MT, Gillespie BW, Warner KE. Patterns of absolute risk of lung cancer mortality in former smokers. *J Natl Cancer Inst* 1993;**85**:457–64.

34 Chung GTY, Sundaresan V, Hasleton P, *et al*. Sequential molecular genetic changes in lung cancer development. *Oncogene* 1995;**11**:2591–8.
35 Fontana RS, Sanderson DR, Taylor WF, *et al*. Early lung cancer detection: results of the initial (prevalence) radiologic and cytologic screening in the Mayo clinic study. *Am Rev Resp Dis* 1984;**130**:561–5.
36 Frost JK, Ball WC, Levin ML, *et al*. Early lung cancer detection: results of the initial (prevalence) radiologic and cytologic screening in the Johns Hopkins study. *Am Rev Respir Dis* 1984;**130**:549–54.
37 Flehinger BJ, Melamed MR, Zaman MB, *et al*. Early lung cancer detection: results of the initial (prevalence) radiologic and cytologic screening in the Memorial Sloane-Kettering study. *Am Rev Respir Dis* 1984;**130**:555–60.
38 Mulshine JL, Scott F. Molecular markers in early cancer detection. New screening tools. *Chest* 1995;**107**:280–6s.
39 Mao L, Hruban RH, Boyle JO, *et al*. Detection of oncogene mutations in sputum precedes diagnosis of lung cancer. *Cancer Res* 1994;**54**:1634–7.
40 Smit EF, Groen HJM, Splinter TAW, *et al*. New prognostic factors in resectable non-small cell lung cancer. *Thorax* 1996;**51**:638–46.
41 Kanters SDJM, Lammers J-WJ, Voest EE. Molecular and biological factors in the prognosis of non-small cell lung cancer. *Eur Respir J* 1995;**8**:1389–97.
42 Harpole DH, Hernden JE, Wolfe WG, *et al*. A prognostic model of recurrence and death in stage 1 non small cell lung cancer utilizing presentation, histopathology, and oncoprotein expression. *Cancer Res* 1995;**55**:51–6.
43 Xu HJ, Quinlan CD, Davidson AG, *et al*. Altered retinoblastoma protein expression and prognosis in early stage non small cell lung carcinoma. *J Natl Cancer Inst* 1994;**86**:695–9.
44 Slebos RJC, Kibbelaar RE, Dalesio O, *et al*. K-ras oncogene activation as a prognostic marker in adenocarcinoma of the lung. *N Engl J Med* 1990;**323**:561–5.
45 Kern JA, Slebos RJC, Top B, *et al*. C-erbB-2 expression and codon 12 K-ras mutation both predict shortened survival for patients with pulmonary adenocarcinomas. *J Clin Invest* 1994;**93**:516–20.
46 Loew SW, Ruley HE, Jacks T, *et al*. p53-dependent apoptosis modulates the cytotoxicity of anticancer agents. *Cell* 1993;**74**:957–67.
47 Fisher DE. Apoptosis in cancer therapy: crossing the threshold. *Cell* 1994;**78**:957–67.
48 Rosell R, Li S, Anton A, *et al*. Prognostic value of K-ras 12 genotypes in patients with advanced non small cell lung cancer receiving carboplatin with either intravenous or chronic oral dose etoposide. *Int J Oncol* 1994;**5**:195–8.
49 Fujiwara T, Cai DW, Georges RN, *et al*. Therapeutic effect of a retroviral wild-type p53 expression vector in an orthotopic lung cancer model. *J Natl Cancer Inst* 1994;**86**:1458–62.
50 Cordon-Cardo C. Mutation of cell cycle regulators. Biological and clinical implications for human neoplasia. *Am J Pathol* 1995;**147**:545–55.
51 Georges RN, Mukhopadhyay T, Zhang Y, *et al*. Prevention of orthotopic human lung cancer growth by intratracheal instillation of a retroviral antisense K-*ras* construct. *Cancer Res* 1993;**53**:1743–6.
52 Hynes NE, Beerli R, Graus-Porta D, *et al*. ErbB-2 and EGF receptor as targets for breast cancer therapy. *Proc Am Ass Cancer Res* 1995;**36**:690–1.