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. 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. 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.

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. 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, $10^{-3}$–$10^{-7}$ per gene per generation, there being $10^{14}$ cells in the body. 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 $1 \times 10^{-22}$—that is, negligible. 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. 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 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 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.
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. 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). 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 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 mutation in DNA repair processes. Mutator class, mutator genes, are involved prior to mutagenesis and cell cycle controls. The third class, mutator genes, are involved prior to mutation in DNA repair processes. Mutator gene inactivation is recessive, like tumour mutation in DNA repair processes. Mutator class, mutator genes, are involved prior to mutagenesis and cell cycle controls. The third class, mutator genes, are involved prior to mutation in DNA repair processes. Mutator gene inactivation is recessive, like tumour mutation in DNA repair processes. Mutator class, mutator genes, are involved prior to mutagenesis and cell cycle controls. The third class, mutator genes, are involved prior to mutation in DNA repair processes. Mutator gene inactivation is recessive, like tumour mutation in DNA repair processes. Mutator class, mutator genes, are involved prior to mutagenesis and cell cycle controls. The third class, mutator genes, are involved prior to mutation in DNA repair processes. Mutator gene inactivation is recessive, like tumour mutation in DNA repair processes. Mutator class, mutator genes, are involved prior to mutagenesis and cell cycle controls. The third class, mutator genes, are involved prior to mutation in DNA repair processes. Mutator gene inactivation is recessive, like tumour mutation in DNA repair processes. Mutator class, mutator genes, are involved prior to mutagenesis and cell cycle controls. The third class, mutator genes, are involved prior to mutation in DNA repair processes. Mutator gene inactivation is recessive, like tumour mutation in DNA repair processes. Mutator class, mutator genes, are involved prior to mutagenesis and cell cycle controls. The third class, mutator genes, are involved prior to mutation in DNA repair processes.

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.9 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 studies12 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 10⁶ 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 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 cell18 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

<table>
<thead>
<tr>
<th>Site of mutation</th>
<th>Name of mutation</th>
<th>Frequency</th>
<th>Type of mutation</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromosome 3 (short arm) 3p14.2, 3p21.3, 3p25</td>
<td>Fragile histidine triad (FHIT)</td>
<td>SCLC = 100% NSCLC = ~60% (SqCLC = ~100%)</td>
<td>Deletion/loss of heterozygosity (LOH)</td>
<td>Tumour suppressor genes; 3p12.1 codes for a mutator gene</td>
</tr>
<tr>
<td>Chromosome 8</td>
<td>MYC</td>
<td>SCLC 30–40%</td>
<td>Translocation (8;14, 8;22)/amplification</td>
<td>Oncogene transcription factor</td>
</tr>
<tr>
<td>Chromosome 9 (short arm) 9p21</td>
<td>p16 (aka CDKN2)</td>
<td>SCLC &gt;80% NSCLC &gt;50%</td>
<td>LOH/small (&lt;500 kb) deletions</td>
<td>Tumour suppressor gene</td>
</tr>
<tr>
<td>Chromosome 12 (short arm) 12p</td>
<td>k-Ras</td>
<td>Adeno 30–40%</td>
<td>Point mutations</td>
<td>Oncogene p21. GTPase</td>
</tr>
<tr>
<td>Chromosome 14–18 translocation</td>
<td>Retinoblastoma (Rb) gene</td>
<td>SCLC &gt;90% NSCLC less common</td>
<td>Point mutations</td>
<td>Tumour suppressor gene</td>
</tr>
<tr>
<td>Chromosome 17 (short arm) 17p13</td>
<td>Bcl-2 (B cell lymphoma/leukaemia 2)</td>
<td>SqCLC 25% Adeno 10%</td>
<td>Chromosomal translocation</td>
<td>Oncogene, inhibits programmed cell death</td>
</tr>
<tr>
<td>Chromosome 17 (long arm) 17q</td>
<td>p53 (aka TP53)</td>
<td>SCLC = ~75% NSCLC = ~50% (but probably most common single genetic change in all cancers)</td>
<td>Deletion/LOH</td>
<td>Tumour suppressor gene</td>
</tr>
<tr>
<td></td>
<td>c-erb B-2/neu</td>
<td>NSCLC 30–40%</td>
<td>Chromosomal translocation/amplification</td>
<td>Oncogene, transcription factor</td>
</tr>
</tbody>
</table>

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

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**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. 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. 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. 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. 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. For example, p53 mutation has been shown to be a significant independent predictor of death in...
early non-small cell lung cancer. Mutation of the Rb gene area halves predicted survival after resection for stages 1 and 2 non-small cell lung cancer. Expression of k-Ras and NEU oncogenes predicts a poor prognosis in resectable non-small cell lung cancer whilst five year survival is higher for patients with Bcl-2 positive tumours. 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.

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. Expression of the k-Ras oncogene seems to produce a resistance to platinum based compounds and etoposide. Defining the mutations present in premalignant 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 and wild type Rb genes and also an “antisense” (AS) k-Ras gene into tumour cells by this method.

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. 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.

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.

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