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Topical Reviews

Oncogenes and onco-suppressor genes in lung cancer

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Introduction

It has been known for many years that the evolution of cancer is a multi-step process [for review see (1) and references therein]. Insight into the nature of some of the steps has been gained through advances in molecular and cellular biology and it has become apparent that mutations in a limited number of genes which control cell proliferation and differentiation is a key feature of this process. Two categories of genes in particular have been implicated: proto- oncogenes and tumour-suppressor genes [for review see (2)]. Proto-oncogenes become 'activated' to oncogenes through particular mutations so that either the normal protein is overexpressed or a mutant protein is produced. Expression of tumour suppressor genes is believed to restrain cells from unregulated proliferation and inactivation of both homologues releases cells from these controls.

Numerous studies have been undertaken to determine the mutational events that are key to particular cancers. For colon cancer, it has been shown that the same type of tumour can arise through activation of different sets of genes (3,4). This is also likely to be true for other cancers including pulmonary carcinoma.

Lung cancer is one of the commonest cancers in the developed world for both men and women. Prognosis is poor with fewer than 13% of all lung cancer patients living more than 5 yr after diagnosis [for reviews see (5,6)]. With such an appalling mortality it is important to elucidate the genetic changes involved in the pathogenesis of lung cancer and to determine how these changes correlate with clinical features. More detailed information may allow earlier diagnosis, better tumour typing, more focused treatment and improved prognosis.

Lung cancers are divided into two main groups on the basis of their histology (5). The first group, small cell lung cancer (SCLC) accounts for about 25% of all lung tumours, is an extremely aggressive neoplasm, is frequently associated with distant metastases and has the poorest prognosis of all lung tumours. It was formerly treated by surgery, but is now treated primarily with chemotherapy and radiotherapy which give slightly improved survival rates (7). The second group, comprising the remaining 75% of lung cancers, are non-small cell lung cancers (NSCLC). There are several sub-types which are characterized by their tissue of origin: adenocarcinomas which arise in the major bronchi, squamous carcinomas which arise in the squamous epithelium and large cell carcinomas which are largely undifferentiated tumours probably arising from stem or basal cells. NSCLC are treated primarily with surgery and radiotherapy. SCLC and NSCLC are regarded as being separate clinicopathological entities on the basis of their morphology, sensitivity to chemotherapeutic agents and ionizing radiation. They will be treated separately in this review.

Oncogenes in Non Small Cell Lung Cancer

RAS

The K-*ras*, H-*ras* and N-*ras* genes encode highly homologous protein monomers, p21^{ras}, that are located on the cytoplasmic side of the plasma membrane. Their similarity in sequence to G proteins suggests that they may play a role in transmitting proliferation signals from receptors at the cell surface to the nucleus (8). They bind guanosine triphosphate (GTP) and display an intrinsic GTPase activity that is enhanced by interaction with the protein, GAP (9). Ras proteins are thought to be in an active state for signalling when GTP is bound and inactive when the GTP has been hydrolysed to GDP (8). Overexpression of the normal gene is activating *in vitro* (8) as are point mutations in codons 12, 13 or 61 which prevent intrinsic GTP

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hydrolysis presumably by trapping the protein in the active form (10).

Amplification of the *ras* genes is uncommon in NSCLC (11–13). One study could find no evidence for high levels of overexpression of *ras* (14), however, another group found elevated levels of $p21^{ras}$ proteins compared to normal adjacent tissue from the same patient (15–17). The level and frequency varies with the tumour type with about 80% of adenocarcinomas compared to 50% of squamous cell carcinomas displaying increased levels of *ras* p21. The exact nature of the protein(s) detected is unknown because the monoclonal antibody used (Y13-259) in these studies neither distinguishes between H-, K- and N-RAS proteins nor whether the protein is normal or mutant.

Activating mutations in H-*ras* and N-*ras* genes are rare in NSCLCs (11,18) except perhaps in Japan (19). There is now evidence linking mutations in any member of the *ras* family with poor prognosis (20). K-*ras* is mutated in approximately 30% of adenocarcinomas, but this activation is rare in other types of NSCLC (13,21). Several groups have now shown that there is a correlation between K-*ras* mutations in adenocarcinomas and a history of smoking (21–24) with about 30% of smokers compared with 2% of non smokers having G to T transversions at codon 12 (21–23). This type of mutation is consistent with exposure of the lung to carcinogens in tobacco smoke such as benzo[a] pyrene. No correlation was found between K-*ras* mutations and sex, age at diagnosis or tumour stage (21), but mutations in K-*ras* are associated with a very poor prognosis (20,21,25).

MYC

The *myc* gene family encodes at least three proteins c-MYC, N-MYC and L-MYC, of M_r 62–68 kDa. [For review see (26)]. The protein products are located in the nucleus, bind to DNA and associate with at least one other protein, Max (27). The function of MYC proteins has not been established, but they may be transcriptional factors (28). Activation of *myc* is thought to occur by gene amplification or overexpression of the protein, but not by point mutations.

Amplification of the c-*myc* gene is found in about 10% and over expression in about 50% of NSCLCs of all types (15). L-*myc* and N-*myc* appear not to be activated in NSCLC although it has been reported that N-*myc* is amplified in a pulmonary adenocarcinoma cell line (29).

Recent RFLP studies have shown that in Japanese patients with lung cancer, the form of the L-*myc* gene correlates with prognosis, metastasis and the incidence of multiple cancers (30,31). This correlation is particularly strong for adenocarcinoma and squamous-cell carcinoma. However, such correlations were not found in studies of Norwegian patients (32) or of white and black patients (33). The reason for these contradictory results is not known, but may arise from differences in the type of tumour analysed or in the ethnic origin of patients.

ErbB-1

ErbB-1 encodes the epidermal growth factor receptor, a transmembrane glycoprotein of M_r 170 kDa that possesses intrinsic tyrosine kinase activity and is though to play a role in signal transduction [for review see (34)]. Activation is commonly by overexpression of the protein, but not by mutation (35).

Few studies have been undertaken on the expression of *erbB-1* in lung cancer, but it is reported that about 25% of squamous SCLCs overexpress *erbB-1* protein (36).

C-erbB-2

The *c-erbB-2* (*neu*) gene encodes a transmembrane, tyrosine-specific protein kinase, $p185^{neu}$. It is a putative growth factor receptor, related in sequence and structure to the epidermal growth factor receptor (37). The gene is frequently amplified in adenocarcinomas and mRNA and protein levels are frequently elevated in tumours as compared to normal tissue.

Amplification of the *c-erbB-2* proto-oncogene is an infrequent event in lung cancer (38). However, overexpression of $p185^{neu}$ as detected immunohistochemically, is frequently found in NSCLCs – 10/29 adenocarcinomas and 5/16 squamous cell carcinomas (39). In adenocarcinomas, $p185^{neu}$ expression tended to be found in older patients and there was an independent association with short survival times. In squamous cell carcinomas, $p185^{neu}$ expression did not correlate with these parameters.

C-FOS AND C-JUN

c-*fos* and c-*jun* encode nuclear proteins that form a complex, AP-1, which acts as a transcriptional factor for genes possessing a specific DNA recognition site (40). Recently, it was reported that in adeno- and squamous cell lung carcinomas, the level of AP-1 activity in nuclear extracts was elevated in ten out of 13 tumours as compared to their adjacent normal tissues (41). As there is evidence that AP-1 may be involved in signal transduction (42), elevated levels may be oncogenic. It has not been established if changes in the AP-1 levels in a tumour correlate with prognosis.

C-RAF-1

The c-*raf* proto-oncogene encodes a serine/threonine-specific protein kinase, $p74^{raf\text{-}1}$, which is located on the

internal side of the plasma membrane (43). RFLP analyses of 73 unmatched NSCLC tumours showed that there was loss of heterozygosity in 31 suggesting that this gene might play a role in the pathogenesis of the cancer (44).

C-MYB

The c-*myb* proto-oncogene encodes a nuclear protein of 75 kDa which is a transcriptional regulator (45). DNA hydridization studies showed that three out of four adenocarcinomas had lost heterozygosity for c-*myb* (38). Analyses of several NSCLCs have shown defects in RNA transcription. Thus in 11 NSCLC cell lines no c-*myb* transcripts were detected (46). These results may indicate that aberrant c-*myb* expression may play a role in generation of lung cancer.

Onco-suppressor Genes in Non Small Cell Lung Cancer

P53

The p53 gene encodes a 53-kDa nuclear phosphoprotein which has recently been identified as a transcriptional activator (47). High levels of the wild type gene product inhibit growth possibly through acting as a checkpoint for DNA damage at the G0–G1 transition of cell division (48,49). Several mutant alleles with particular single base substitutions encode proteins that confer altered cell growth regulatory properties (48).

Mutations in p53 are the commonest genetic changes detected in several different types of cancers and are a common feature of NSCLCs (50). The frequency varies with the type of pulmonary cancer with about 67% of squamous cell carcinomas and 37% of adenocarcinomas carrying p53 mutations (51,52). Mutations have also been reported in large cell lung cancers, three out of six, but the number of cases studied was low (52,53). No statistically significant correlation has been found between p53 mutations and the age or sex of patients or the histology, clinical stage, or lymph node involvement (51).

G:C to T:A transversions are found in about 50% of NSCLCs (51,54). This is a type of mutation in p53 that is uncommon in other types of human cancer (50). Since one of the components of cigarette smoke is benzo[a] pyrene, a potent mutagen that causes G:C to T:A transversions (55), a correlation between smoking and these particular mutations might be expected. While a study of NSCLCs in Japanese patients showed that there is indeed a statistically significant association of p53 mutations with lifetime consumption of cigarettes (51), an earlier analysis of p53 mutations in American lung cancer patients found a different nucleotide substitution pattern and failed to find any significant correlation between smoking and p53 mutations (52). The reasons for these contradictory results are unknown. One possibility is that there may be differences in the genetic susceptibility to lung cancer in the two populations.

Mutations at many positions along the p53 gene have been found (51,53,56). Codon 273 has been reported to be a 'hot spot' for mutation in American patients with lung cancer, but none of 26 patients carried a mutation at this site in a Japanese study (51).

Immunocytochemical studies have shown higher levels of mutant p53 protein in squamous cell and in adenocarcinomas of the lung than in normal adjacent tissue (17). The higher the level of p53 present, the poorer was the prognosis both with respect to the primary tumour ($P < 0.05$) and to lymph node metastases ($P < 0.005$) (17).

p53 alterations have been detected in preneoplastic lesions of the lung (57,58) suggesting that p53 changes occur in the early stages of lung cancer development. The possibility arises that screening for p53 mutations could form the basis for early detection of lung cancer.

p53 binds to the product of the MDM2 gene (59) which was originally identified as a murine dominant transforming oncogene (60). The MDM2 gene maps to 12q13–14 in man, a chromosomal region that is often altered in sarcomas (61). Amplification of the MDM2 gene is found in over one third of sarcomas (59) and it has been suggested that in cancers where only non-mutated p53 can be detected, functional p53 protein may nevertheless be unavailable to the cell by virtue of being sequestered by excess MDM2 (59). The status of the MDM2 gene in lung cancer will doubtless be the subject of intense investigation.

RB

The retinoblastoma (Rb-1) gene encodes a DNA binding protein of 110 kDa that is thought to be involved in events important in cell division (48). Inactivation of the Rb gene by deletion and loss of heterozygosity have been found in several cancers (48). The status of the Rb-1 gene in NSCLC is not known, but no alterations were found in 20 NSCLC-derived cell lines (62). Anti RB1 peptide antibodies precipitated RB1 protein in eight out of nine NSCLC-derived cell lines, so at least in cell lines RB1 protein is commonly expressed (62).

NM23

The nm23 gene located on the long arm of chromosome 17 at 17p11-q11 (63) encodes a nucleoside diphosphate kinase (65). Low levels of nm23 mRNA and the corresponding protein have been found to

correlate with high metastatic potential in several tumours (66,67). Somatic deletion of an allele of nm23 has been reported in adenocarcinomas of the lung (63), but an investigation into its pronostic value showed there was no correlation between nm23 product and survival rates (68).

Chromosomal Abnormalities

The presence of consistent chromosomal losses or deletions has suggested the involvement of known or candidate tumour suppressor genes at these locations. This has been confirmed by RFLP analysis showing LOH for genes on chromosomes 13q (RB) and 17p (p53) (69).

The karyotype of NSCLC is complex even in cells obtained from tumours prior to chemotherapy (70). In one recent study, chromosomes 1, 3, 6, 7, 8, 11, 13,15, 17 and 19 were found to carry non random rearrangements (70). The most frequently rearranged bands being 1p13, 3p13, 8p11–q11, 15p11-q11 and 17p11 each of which appeared in 8-14/30 samples. In a different study RFLP deletion analyses of DNA from 53 primary NSCLCs showed loss of heterozygosity (LOH) to be a frequent event on the long arms of chromosomes 1 (37%), 2 (31%), 5 (30%), 8 (31%), and 13 (32%) and the short arms of chromosomes 3 (54%) and 17 (62%) (71). LOH on chromosomes 3p and 17q were more frequent in squamous- than in adenocarcinomas.

Deletions in the short arm of chromosome 3 occur in over 75% of NSCLCs (72–74). The regions, 3p21.3 and 3p14.1–21.1 were preferentially deleted in adenocarcinomas and loss of heterozygosity at these regions was seen more frequently in poorly- or undifferentiated- than in well-differentiated tumours (74). In addition, the frequency of deletion was higher in stage III than in stage I or II tumours ($P = 0{\cdot}043$). No correlation was found with parameters such as smoking status, node size and distant metastases. In all squamous cell lung carcinomas analysed, there was a deletion at one or both of the regions. These results suggest onco-suppressor genes may be located at 3p21.3 and 3p14.1–21.1 and that they may be involved in the progression of the tumours.

Changes in Small Cell Lung Carcinoma

Mutations in *ras* genes are essentially absent in SCLC – 0/42 (75), 0/12 (21) and elevation of *ras* protein expression is rare and not of statistical significance ($P = 0{\cdot}07$) (17).

Initial studies on *myc* expression in SCLC were performed on cell lines and found evidence for c-*myc* or N-*myc* gene amplification (16,17) in about 30% of cell lines. c-*myc* amplification was seen only in patients who had undergone chemotherapy and a correlation between c-*myc* amplification in treated patients and shorter survival times was found (17).

Gene amplification of all three members of the *myc* family have been observed in primary SCLC (78,79). In one study 19/26 patients with metastatic SCLC had amplification of either c-*myc* or N-*myc* and there was no association between mutations and variant morphology of tumours. Metastases and tumours had similar copy numbers of the genes suggesting that *myc* amplification was an early event. There does not appear to be a correlation between the degree of *myc* gene amplification in primary tumour and survival time (79). However, it is necessary to interpret these results with caution as the survival time of SCLC patients after diagnosis is so short anyway.

In accord with data from cell lines, amplification of *myc* DNA occurs more frequently in patients who have undergone chemotherapy, but cell lines established from SCLCs before and after chemotherapy did not alter their status of *myc* gene copy number (80). Thus, it is not clear if chemotherapy can actually cause *myc* gene amplification.

Elevated expression of *myc* has been observed in five of six patient tumours, but the level of expression did not necessarily correlate with the degree of *myc* gene amplification (81). In another study of 15 primary biopsies from patients who had not undergone chemotherapy, N-*myc* RNA levels were increased in six cases. Increased expression of N-*myc* correlated with a poor subsequent response to chemotherapy, rapid tumour growth and short survival times ($P < 0{\cdot}01$) (82). The basis for these observations is not known.

In a study which included 21 patients with SCLC, immunohistochemical analyses showed that c-MYC protein was over-expressed in 4 patients. While the sample number is low this may be of statistical significance ($P < 0{\cdot}05$) (83). The status of c-*myc* amplification did not correlate with prognosis of the patients.

RFLP analyses of 84 primary human lung carcinomas indicated LOH for the c-*raf*-1 locus in five of five informative matched normal and tumour SCLC samples and in 42 of 42 unmatched SCLC tumours (44).

Onco-suppressor Genes in SCLC

Mutations in p53 are present in over 75% of SCLCs (54,84–86). In one study, loss of one p53 allele and mutation of the other was found in 16/16 stage III–IV tumours and 3/6 stage I–II tumours (85). In addition, the allelic loss and/or mutation found in primary

tumours was also observed in metastases in distant organs suggesting that alterations to p53 are early events in the pathogenesis of SCLC.

Structural abnormalities within the Rb gene have been detected in one of eight (13%) primary SCLC tumours and 4/22 (18%) SCLC lines (87,88). Absence of RB mRNA and p105 RB protein however, is common in SCLC lines (87,89).

Deletions of the short arm of chromosome 3 are common events in SCLC (90,91) with three distinct regions between 3p21–25 being involved (73). Four genes, ACY1, APEH, PTPG and D8 have been cloned from this region and their expression in SCLC cell lines compared with levels in normal lung (92–95). All are under-expressed in at least one cell line, but data are so far insufficient to establish a role for any as a tumour suppressor gene in SCLC.

LOH in the tumour suppressor genes, MCC (mutated in colon cancer) and APC (adenomatous polyposis coli), have been implicated in the pathogenesis of several cancers (96–98). Both genes are located on the long arm of chromosome 5 (5q21) a region that is often deleted in SCLC (99). Over 80% of SCLCs show allelic deletion of these genes raising the possibility that they are involved in tumourigenesis (100).

Subtractive hybridization cloning of sequences in a lung cancer and normal cell line led to isolation of three sequences present in normal, but not in the tumour-derived DNA (101). Since one sequence, del-118, was also deleted in a freshly isolated lymph node metastasis from an adenocarcinoma, it is a candidate tumour suppressor gene for lung tumours.

Concluding Remarks

Regardless of the tissue or origin, the development of a cancer cell from a normal cell involves a series of genetic changes that contribute to a loss of normal growth control mechanisms. For lung cancer we are still far from understanding what these events are. Significant progress has been made recently in determining the status of oncogenes such as *ras* and *myc* and onco-suppressor genes such as p53 whose role is considered to be important in the multistep process of carcinogenesis. Consistent chromosome deletions may facilitate identification of more tumour suppressor genes and it is worth noting that microscopically visible deletions cover several million base pairs so several tumour suppressor genes could be located 'close' together. The expression in lung cancer patients of neural cell adhesion molecule and blood group antigen A has been linked to favourable prognosis of patients (102,103). This presents another promising line of investigation.

Further detailed studies are required to probe relationships between mutations and factors such as diagnosis: clinical parameters: genetic predisposition and prognosis.

In addition, it will be necessary to try to order the specific genetic alterations in terms of time as this may allow the development of new diagnostic tools, and treatments for lung cancer.

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