The molecular genetics of human lung cancer

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ABSTRACT: With the development of molecular biological techniques the search for genetic alterations in cancer cells has resulted in the beginning of a molecular description of cellular transformation. Most of these genetic changes occur in genes which have a role in the control of cellular growth and development, the proto oncogenes. In the last decade, it has become clear that the myc and ras oncogene families are important in the carcinogenesis of human lung cancers. The myc oncogenes are usually found to be altered in small cell lung cancer (SCLC), and these alterations appear to correlate with rapid growth and progression. Mutations in the Kras gene are specific for adenocarcinoma, a subclass of non small cell lung cancer (NSCLC). Kras gene mutations are closely associated with tobacco smoking, since all were found in adenocarcinomas from patients with a history of smoking. The erbB oncogene, which encodes the epidermal growth factor receptor, is often highly expressed in epidermoid carcinomas. The roles for other oncogenes, such as ras or myb, as well as those of "suppressor" genes remain to be investigated, but may be of paramount importance. The study of alterations in proto oncogenes may aid in the (sub)classification and diagnosis of lung cancer, and may yield useful prognostic information in the near future.

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Most human lung cancers can be classified as one of four major types: small cell carcinoma, epidermoid carcinoma, adenocarcinoma, and large cell carcinoma. The latter three types are also referred to as non small cell lung cancer (NSCLC) [1]. Clinically, the distinction between small cell lung carcinoma (SCLC) and non small cell lung carcinoma is important because SCLC is treated mostly with chemotherapy while the treatment of choice in limited NSCLC is surgery. For all types of lung cancer, however, treatment results are disappointing, and the outlook for patients whose tumors cannot be completely resected remains grim. After curative resection 5 yr survival is about 30% in NSCLC, but the majority of the patients are already inoperable at time of presentation and their 2 yr survival is only a few percent [2].

It is in this context that one might hope that a better understanding of the biology of human lung cancer will eventually lead to urgently required new treatment strategies. The study of cellular genes involved in human cancer has progressed rapidly since viral genes from acutely transforming animal retroviruses were shown to have cellular counterparts [3]. When introduced into mouse fibroblasts in vitro, the genes were able to transform these cells [4] and were called proto oncogenes. More than 40 different proto oncogenes have been identified to date [5–7]. Most of these genes are thought to be of critical importance in the regulation of normal cellular growth and development. In the normal situation, the expression of proto oncogenes is strictly regulated and the gene products are able to fulfill their essential roles in cellular physiology. The name "proto oncogenes" is, thus, more informative about the way in which these genes have been discovered than about their normal functions in the cell. It is only when these genes are activated by specific structural alterations or by a disturbance of their genetic expression that their relationship with tumorigenesis becomes evident. After such an activation the product, which is now called an oncogene, acquires transforming properties.

Extensive investigations in this decade have revealed several mechanisms by which a proto oncogene can become activated, and some of these changes have also been found to have a role in the development of primary human malignancies. One of the best studied mechanisms by which a proto oncogene can become activated is by amplification, a process in which the proto oncogene region in the deoxyribonucleic acid (DNA) is replicated several times within the cellular genome [8]. This process usually results in an increased amount of messenger ribonucleic acid (RNA) and hence of proto oncogene encoded protein within the cell. If the amount of amplification is substantial, the over-expressed normal proto oncogene product may exert transforming activity.

Chromosomal translocation is another mechanism by which a proto oncogene may become activated. In this process, which is a common event in genetically
unstable tumour cells, parts of chromosomes are deleted or translocated to other chromosomes. This may either result in loss of DNA sequences which control genetic expression of other genes or may induce reciprocal translocation of two different genes. The latter case may result in a combination of two protein encoding DNA segments and hence lead to an aberrant fusion protein which may exert transforming activity.

A more subtle change which can activate a proto oncogene is that of a single base pair alteration, a point mutation, in the protein encoding domain of the gene. This mechanism is seen in the ras family of proto oncogenes (see below). If a point mutation occurs at a specific position in the DNA sequence, the resulting altered ras protein acquires transforming activity.

In table 1 a summary is given of the proto oncogenes which have been reported to be activated in one or more type of human lung cancer. Of these genes the best studied examples are the cmyc and ras genes, genes that were amongst the first proto oncogenes to be discovered. Apart from these, other genes might be involved in the carcinogenesis of human lung cancer e.g. the erbB, myb and raf genes which appear to be altered in their expression in human lung cancers.

An exciting new concept in the molecular genetics of cancer is that of the “suppressor” genes or “anti oncogenes” which are thought to suppress other genes which, if uninhibited, may contribute to malignant transformation. Chromosomal deletion may result in loss of such a gene which may disclose a null mutation in the other allele. Deletion in the short arm of chromosome 3 which has been described for human lung cancer might reflect the loss of a “suppressor” gene.

The novel insights into the pathogenesis of human (lung) cancer generated by the discovery of the cellular proto oncogenes, should ultimately find clinical applications. Firstly, the detection of activated oncogenes in malignancies might lead to a better (sub)classification of certain types of tumours. Secondly, activated oncogenes might serve as targets for newly developed antineoplastic drugs or for novel genetic modalities of therapy. This paper reviews the detection of activated cellular oncogenes, their apparent role in human lung cancer, and their possible clinical relevance.

### The myc proto oncogenes

There is little doubt that proto oncogenes of the myc family have a definite role in the pathogenesis of human cancer. This family consists of at least three closely related genes which are called cmyc, Nmyc, and Lmyc. All encode nuclear proteins with DNA-binding properties involved in the regulation of the cell cycle [6]. Activation of cmyc is invariably found in Burkitt lymphoma, in which cmyc is translocated to one of the immunoglobulin loci which results in an aberrant expression of the gene [9]. Evidence for the direct involvement of the translocation in the pathogenesis of Burkitt lymphoma comes from studies with transgenic mice, in which the integration of an activated cmyc gene in the genome results in a high incidence of lymphomas [10, 11]. The Nmyc gene is found amplified and over-expressed in neuroblastoma cell lines [12], whilst amplification of this gene is also frequently observed in primary neuroblastomas [13]. In this tumour, amplification of Nmyc is an early event associated with poor prognosis and hence over represented in the advanced stage of the disease [14]. To date, this is one of the few examples of a clinically applicable result of oncogene research. The third gene, Lmyc, was first detected in an SCLC cell line [15]; its genetic activity is regulated by a complex mechanism [16].

In lung cancer, the patterns of activation of the myc genes are more complicated and less clear than in Burkitt lymphoma or neuroblastoma. Most work has been carried out with cell lines derived from SCLCs, which can now be established with relative ease, and are thus available for cytogenetic and molecular genetic studies. Amplification of cmyc in five of eight SCLC cell lines was first reported by the group of Little et al., at the National Cancer Institute (NCI) [17]. Interestingly, all cmyc amplifications were found in cell lines which differed from the "classical" SCLC in their biochemical properties and morphology and are referred to as "variant" SCLC. In later studies at NCI and in other laboratories, the association between an amplified cmyc gene (or sometimes high cmyc expression without amplification) and SCLC cell lines could be confirmed [18, 19]. In SCLC cell lines, N- and Lmyc do sometimes appear to be amplified also [20]. At NCI, the analysis of 44

### Table 1. Properties of cellular proto oncogenes involved in lung cancer

<table>
<thead>
<tr>
<th>Oncogene</th>
<th>Mechanism</th>
<th>Location</th>
<th>Function</th>
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<tbody>
<tr>
<td>cmyc</td>
<td>Amplification</td>
<td>Nucleus</td>
<td>DNA-binding</td>
</tr>
<tr>
<td>Nmyc</td>
<td>Amplification</td>
<td>Nucleus</td>
<td>DNA-binding</td>
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<tr>
<td>Lmyc</td>
<td>Amplification</td>
<td>Nucleus</td>
<td>DNA-binding</td>
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<tr>
<td>Hras</td>
<td>Point-mutation</td>
<td>Inner</td>
<td>GTP-binding</td>
</tr>
<tr>
<td>Kras</td>
<td>Amplification</td>
<td>Cell membrane</td>
<td>Growth factor receptor</td>
</tr>
<tr>
<td>Nras</td>
<td>Deletion</td>
<td>Nucleus</td>
<td>DNA-binding</td>
</tr>
<tr>
<td>erbB</td>
<td>Rearrangement</td>
<td>Cytoplasm</td>
<td>Serine/threonine kinase</td>
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DNA: deoxyribonucleic acid; GTP: guanosine triphosphate.
SCLC cell lines revealed five c-myc, four N-myc, and four \(L\)-myc amplifications [21], whilst a British study demonstrated four \(N\)-myc and two \(L\)-myc amplifications in eight SCLC cell lines [19]. Amplifications of \(N\)- and \(L\)-myc were not restricted to the “variant” SCLC cell lines, but were also found in “classical” SCLC cell lines.

Cell lines of the “variant” type have a different morphology in culture, faster growth rates, higher cloning efficiency in soft agar, resistance to radiation in vitro, and a decreased expression of neuroendocrine markers such as L-dopa decarboxylase, neuroendocrine granules, and peptide hormones related to bombesin [22, 23]. There is evidence that comparable changes occur in patients during the course of the disease.

In about 20% of all SCLCs patients a change to a non small cell histology is apparent during tumour progression [24]. Recent observations suggest that small cell carcinomas with a distinct admixture of large cells are even more aggressive than other SCLCs [25]. Furthermore, amplifications of \(m\)-yca genes appear to be more frequent in tumours which relapse after chemotherapy than in tumours from untreated patients [21].

An obvious question arising from these data is whether or not the enhanced expression levels of \(m\)-yc are the direct cause of the “variant” SCLC cell line phenotype. This was investigated by the introduction of an active \(c\)-myc gene into a “classical” SCLC cell line [26]. As a result of this transference, the cell line acquired some, but not all “variant” characteristics: altered morphology, faster growth rate and a resistance to radiation, but no loss of neuroendocrine markers. Thus, it is clear that \(c\)-myc over-expression in SCLC cell lines is responsible for some of the “variant” characteristics, but that its amplification is not the only step in the progression from the “classical” to the “variant” phenotype.

Although frequent in SCLC cell lines, amplification of the \(c\)-myc proto oncogene in fresh tumour specimens has only occasionally been found. The group from NCI did not demonstrate \(c\)-myc amplifications in 20 tumour samples obtained at autopsy [18], whilst Woon et al. [27] detected two \(c\)-myc and three \(N\)-myc gene amplifications in 96 DNA preparations of paraffin embedded tissues from 45 patients. Furthermore, when SCLC cells are cultured for only a limited period (up to a few months) \(m\)-yc gene amplifications are rare: an amplified \(c\)-myc or \(N\)-myc could be detected in none of ten SCLC cell lines [28].

It can be concluded from these data that amplification of \(m\)-yc family genes does not appear to be a primary event in the pathogenesis of SCLC. Amplification of these genes may render a growth advantage to an already transformed cell, which can subsequently become the dominant population in a tumour. Indications for the relevance of such a mechanism is the increase in \(c\)-myc copy number which was observed when an SCLC cell line was maintained as a xenograft in nude mice [18].

The clinically important question, whether or not \(m\)-yc amplified tumours have a worse prognosis, was investigated at NCI: 44 cell lines derived from 227 patients with SCLC were studied [21]. It was found that patients whose tumours could successfully be cultured in vitro had a significantly poorer prognosis than others. An amplified \(m\)-yc gene was found in only 2 out of 19 cell lines derived from untreated patients (1 \(N\)-mlyc and 1 \(L\)-mlyc), whilst \(m\)-yc amplifications were present in cell lines from 11 out of 22 patients who had relapsed after chemotherapy (5 \(c\)-myc, 3 \(N\)-myc, and 3 \(L\)-myc). Those patients from whom amplified \(m\)-yc containing cell lines had been established had a poorer survival (and thus presumably more aggressive tumours) than others.

In another study, 7 out of 15 primary SCLCs had an increased expression of \(N\)-myc as determined by in situ hybridization on paraffin embedded, formalin fixed specimens [29]. These patients had a shorter survival and were less responsive to chemotherapy than patients with low \(N\)-myc expression. In another study, a good response to chemotherapy in 2 out of 3 SCLCs which had \(c\)-myc gene amplifications was reported [27]. Thus, although frequent in SCLC cell lines, only a minority of newly diagnosed SCLCs have amplified \(m\)-yc genes. It therefore seems likely that \(m\)-yc gene amplifications render a growth advantage to already transformed cells, particularly under conditions of cell culture and in patients who received chemotherapy.

Since establishing cell lines from NSCLCs is more difficult than from SCLCs, only a limited number of cell lines have been available for the study of proto oncogene activations so far. In two NSCLC cell lines derived from two independent giant cell carcinomas of the lung an amplified \(c\)-myc gene, accompanied by gene rearrangement was detected [30].

In one adenocarcinoma cell line a \(c\)-myc amplification was detected, while four NSCLC cell lines investigated had normal \(c\)-, \(N\)-, and \(L\)-myc gene copy numbers [31]. One case of a large cell lung carcinoma xenograft maintained in nude mice has been described, which contained an amplified \(c\)-myc gene together with a mutationally activated \(Kras\) gene [32].

In primary NSCLC, aberrations of the \(m\)-yc proto oncogenes have also been described, but do not appear to be frequent. Amplification of \(c\)-myc has been detected in 1 out of 3 adenocarcinomas [33], 1 out of 2 broncho alveolar carcinomas [34], 3 out of 26 NSCLCs [35], and none of 24 NSCLCs [36]. In our series of DNAs, we detected two cases of \(c\)-myc amplification, but no \(N\)- or \(L\)-myc amplifications in 52 specimens of untreated NSCLC [31]. Another study could not demonstrate any \(c\)- or \(L\)-myc amplification in 25 NSCLCs tested, but revealed a highly amplified \(N\)-myc gene in one adenocarcinoma [37]. The clinical or biological significance of amplified \(m\)-yc genes in clinical samples is not clear. Since these aberrations are only very infrequently found, it is conceivable that they do not have an important role in the early carcinogenesis of NSCLC. Amplification of \(m\)-yc genes occurring at a later stage, for instance during treatment of the tumour, cannot be excluded since comprehensive studies of this question with autopsy specimens have not yet been performed. Furthermore, the known \(m\)-yc genes may not be the only ones involved in lung carcinogenesis: some reports indicate that additional members of the \(m\)-yc proto oncogene family will be identified in the future [18, 37]. Although knowledge of \(m\)-yc gene amplification or over-expression could possibly be used as an
additional prognostic test, the clinical implications of these findings appear to be limited at present. Since amplification or over-expression of the myc genes seems to be an event associated with progression and not with primary transformation, these genes do not appear a suitable target for therapeutic strategies. Even if the suppression of these genes or their products would be successful, this might only lead to reversion to a less undifferentiated phenotype, which would still constitute a lethal disease. A single study has attempted to exploit the presence of myc overexpression in lung cancer for diagnostic imaging [38]. A radio iodine labelled monoclonal antibody against the cmyc gene product was used. In 12 out of 14 lung cancer patients good tumour localization was possible, including 2 out of 3 epithelial carcinomas and 2 out of 3 adenocarcinomas. The scans only detected large lesions, probably because a degree of necrosis was necessary to make some of the nuclear proteins accessible for the antibody.

The ras proto oncogenes

In the search for transforming DNA sequences, genes of the ras family have frequently been encountered. This family includes at least three closely related genes which are called H-, K-, and Nras [39]. The first two ras genes were isolated from the Harvey and Kirsten murine sarcoma viruses, whilst Nras was discovered in a neuroblastoma. Homologues of ras can be found in many different eukaryotic organisms, from yeast to mammals, suggesting a fundamental role in cellular processes. In humans, all three genes encode closely related 21 kD proteins (called p21) which are located at the inner cell membrane, which can bind guanosine triphosphate (GTP) and guanosine diphosphate (GDP), and which possess GTPase activity [39, 40]. The proteins share these biochemical and also some structural properties with G proteins, which have a key role in the transduction of signals from membrane bound receptors to the adenylate cyclase pathway. Thus, it has been postulated that the ras p21 proteins may serve as mediators of external signals, such as those generated by growth factors [41].

Although the exact biological function of the ras p21 proteins is still not completely clear, their oncogenic activation does appear to depend on the loss of their GTPase activity. Activating point mutations in codons 12, 13 and 61, all in or near the GTP-binding domain [42], reduces the p-21ras GTPase activity by about 100-fold. Without this activity, the biologically active GTP bound state persists even without an external stimulus, and a signal is transduced that is, in fact, not there. Another mechanism of activation of the ras genes consists of enhanced expression, which in human tumours is usually the result of gene amplification [43]. This mechanism of activation is still poorly understood, but it may be hypothesized that enhanced levels result in sufficient p21 in an active (GTP bound) state to induce malignant transformation without altering the ratio between active and non-active p21 molecules. Expression of ras genes has been encountered in a wide variety of normal adult tissues as well as in many tumour types [44–46]. In human malignancies, mutationally activated ras genes are found in about 15% of all cases, whilst the incidence of amplified ras genes appears to be about 1% [40]. Mutations in ras genes are particularly frequent in colorectal carcinomas [47], haematological malignancies [48], and lung carcinomas [49]. Recently, pancreatic carcinomas were shown to contain point mutated Kras genes in more than 90% of all tumours [50].

In human lung cancer, the ras mutations were first reported to occur in cell lines derived from NSCLC [51, 52]. In a recent review a total of 13 lung cancer cell lines with ras mutations were listed [39]. Most of these cell lines were derived from lung adenocarcinomas harbouring a mutation in codon 12 of Kras.

In uncultured human lung carcinomas, a specific mutation in codon 12 of Kras could be detected using an SstI restriction fragment length polymorphism (RFLP) [53]. In this case, the SstI restriction endonuclease can only recognize and cut the mutated sequence. The enzyme thus generates a specific DNA fragment which is not generated when only the normal DNA sequence is present. With this technique, only one of the possible point mutations can be detected, one that appears to be infrequent in human lung cancer [54].

Recently, a method employing the polymerase chain reaction (PCR) followed by oligonucleotide hybridization, was developed that allows routine screening of tumour DNA samples for the activation of ras genes by point mutation [55]. Using this method, a set of 36 human NSCLC specimens obtained at thoracotomy was analysed by our group in collaboration with Rodenhuis et al. [56]. Of these, only 5 specimens (14%) had an activated ras gene, all having a point mutation in codon 12 of Kras. This frequency was in good agreement with other studies including the results obtained with NSCLC cell lines. Unexpectedly, however, all mutations were found in 5 of the 10 adenocarcinomas, whilst none were detected in either the epidermoid or large cell carcinomas investigated. This finding raised the question as to whether the Kras oncogene might have been activated by amplification in the 5 mutation negative adenocarcinomas, or whether as yet unknown factors had complemented for the Kras mutation. Subsequent Southern blot analysis revealed that the Kras proto oncogene was amplified in none of the primary lung tumours, a finding which is in agreement with the results of an earlier study on lung carcinomas [36].

One conclusion at this point was that the Kras point mutation was an essential step in the carcinogenesis of lung adenocarcinoma, and that in the mutation negative tumours another similar event had taken place. An alternative explanation for our observations could be that the Kras mutation was a secondary event which had been induced in one or more already transformed cells, which had subsequently overgrown the rest of the tumour population.

In an effort to find clues to answer these questions, the clinical information on these 10 adenocarcinomas were examined, but no histological differences were observed between the two groups. One striking difference, however, was noted between the smoking histories: all Kras
mutation positive tumours were from heavy smokers, 
whilst in the mutation negative group three patients had 
never smoked or had stopped smoking long before. A 
second, larger series of lung adenocarcinomas essentially 
confirmed these results: mutations being found in about 
one third of all adenocarcinomas, all from patients with 
a history of smoking [49].

These data, taken together with the association of 
tobacco smoking with the occurrence of lung tumours 
[57], lead to the attractive hypothesis that the Kras 
mutations in the tumours of smokers have been induced 
by some carcinogenic component in tobacco smoke. 
Indeed, ras mutations are known to represent links be-
tween carcinogen exposure and oncogene activation: ip 
experimental animals mutations in ras genes can be 
induced by a wide variety of carcinogens and are asso-
ciated with the occurrence of several tumour types [58].

The association between carcinogen induced Kras 
mutations and adenocarcinoma of the lung is also known 
from animal studies. In 12 out of 14 mice, Kras codon 
12 point mutation containing lung tumours could be 
induced by benzo(a)pyrene [59], a potent carcinogen known 
to be one of the components of tobacco smoke [60]. 
Other carcinogens may also activate Kras in lung tu-
mours of experimental animals [58, 61]. Exposure to 
radioactive plutonium caused lung tumours containing 
mutated Kras in all of eight dogs [62]. In some inbred 
mice strains the occurrence of lung adenocarcinomas is 
closely correlated with an aberrant Kras gene [63], whilst 
in 6 out of 8 transgenic mice with a point mutated Hras 
gene, lung adenocarcinomas were found [11]. The latter 
findings suggest that the normal tissue specificity can be 
overcome by introducing a constitutive promoter in the 
transgene, and that in this case the expression of the 
mutated Hras p21 protein alone is sufficient to induce 
tumour development.

Although these results may not be directly applicable 
to the situation in humans it is tempting to speculate that 
carcinogens in tobacco smoke are the inducers of the 
Kras point mutations and that, by a still unknown me-
chanism, these mutations specifically lead to tumours of the 
adenoacarcinoma type. The size of our second series of 
lung tumours permitted us to examine the correlations 
between other clinical parameters and the Kras point 
mutations. Statistical analysis revealed that the Kras 
positive adenocarcinomas were significantly smaller and 
were less likely to have spread to the local lymph nodes 
at the time of diagnosis than negative ones [49]. Due to 
the still short follow up the relevance of this finding 
remains to be assessed.

Proto onucogenes other than myc or ras

Activational patterns of other proto onucogenes than the 
myc or ras genes with potential roles in the carcinogene-

cis of human lung cancer are now beginning to emerge. 
One major candidate is the erbB proto onucogene, which 
encodes the epidermal growth factor receptor (EGFR). 
This gene can be activated by either amplification or 
arrearangement, both of which have been encountered in 

human glioblastomas [64]. Over-expression of erbB has 
been described in 6 out of 11 lung carcinoma cell lines, 
of which two, derived from adenocarcinomas, had an 
amplified erbB gene [65]. In uncultured NSCLCs, the 
situation is unclear: amplification of erbB was found in 
5 out of 47 NSCLCs, whilst 20 out of 47 showed loss of a 
genomic fragment encoding the tyrosine kinase domain 
of the EGFR [66]. Others have found amplification of 
erbB in 6 out of 27 NSCLCs [35], whilst in a further 
study of 10 epidermoid carcinomas tested two contained 
an amplified erbB gene, but none of eight adenocarcino-
mas [67]. In our series of DNAs, which included 13 
epidermoid, 21 adenocarcinomas, 9 large cell carcinomas, no 

early erbB copy numbers were detected [31]. Others 
have reported absence of amplification in 28 lung carci

nomas of all four types [37], or in 9 squamous and 15 
adenoacarcinomas [68]. The latter study, however, reports 
over-expression of the gene in 4 out of 14 NSCLCs, 
indicating that in these tumours the regulation of erbB 
gene expression had been altered. Immunohistochemical 
studies of EGFR expression showed a strong staining 
in 11 out of 11 epidermoid lung carcinomas but in only 
one out of 8 adenocarcinomas and none of 2 SCLCs [69].
The correlation between epidermoid carcinomas and 
EGFR staining, however, was less obvious in a subse-
quent study: 28 out of 36 epidermoid carcinomas were 
positive when the same monoclonal antibody was em-
ployed [70]. Fifteen out of 15 SCLC were negative in 
this study. Another large study of NSCLC specimens 
revealed enhanced protein levels in 39 out of 109 
NSCLC tumours, of which 27 were epidermoid carcinoma
mas [67]. Thus, it seems that activation of the erbB gene 
by amplification, accounts for only some of the elevated 
EGFRs in NSCLC, and that an altered regulation of 
the expression of the gene may be at least an equally 
important activation mechanism in human lung carc

inomas.

Further investigations should reveal whether EGFR 
positive tumour cells differ in their clinical behaviour 
from EGFR negative ones. The nuclear protein encoding 
proto oncogene myb has been found to be altered in 2 
lung adenocarcinomas as a result of loss of one of the 
two alleles normally present in the genome, or as a result 
of amplification of the gene in another adenocarcinoma 
[35]. One study reported over expression of the myb gene 
in SCLC [71]. Another proto oncogene, called ras, is 
transcriptionally active in SCLCs [20]. The significance 
of these findings remains to be investigated.

Tumour suppressor genes

Activation of cellular oncogenes is only one of the 
possible events which may lead to tumorigenesis, and 
other genetic alterations may be equally important [72]. 
In several hereditary cancers, loss of genetic information 
can predispose for the development of malignancy. The 
best documented example of such a situation is the case 
of familial retinoblastoma. In patients with this disease, 
a germ line mutation is present which affects one of the 

two alleles of the retinoblastoma (Rb) gene. The
likelihood of a somatic mutation occurring in the other allele of one of the developing retinal cells is high, and such an event is followed by the development of a retinal tumour at an early age [73]. The Rb locus is now known to reside on the long arm of chromosome 13, and cloned DNA sequences from this area are thought to represent a tumour "suppressor" gene [74]. The Rb gene is expressed in many tumour cells and also in foetal retina, but not in retinoblastomas. Nevertheless, it remains to be shown that the gene can revert some of the malignant properties of retinoblastoma cells.

In human lung cancer, several studies have reported non-random chromosomal deletions and the molecular analysis of the involved chromosomal regions is underway. In SCLC, a consistent deletion in the short arm of chromosome 3, with a shortest region of overlap at 3p 14 distal p23, has been reported by several authors [19, 28, 75, 76], although this abnormality was not apparent in all cell lines studied [28, 76]. Submicroscopic evidence for 3p deletions can be obtained by using molecular cloned DNA fragments as probes for this region. The most widely used for this purpose is pH2H3 [77]. This probe detects a restriction fragment length polymorphism (RFLP) between the two alleles of the locus in a heterozygous individual. If only one of the two alleles is present in the tumour DNA, one of the normally present fragments on a DNA blot will be lost. Several authors have used the pH3H2 probe for the analysis of DNA isolated from normal lymphocytes and from the tumour of the same patients. This work revealed that the defect is invariably present in SCLC [78-80], but somewhat diverging results have been reported for NSCLC.

Some studies suggest allelic loss in some but not all tumours [78, 81], another study suggests a tight association similar to that in SCLC [79]. The putative tumour "suppressor" gene might, thus, be important not only for SCLC but also for at least some cases of NSCLC. Furthermore, in all tumours studied so far the deletion was found to be present in only one of the two chromosomes. It is at present not clear whether or not other, more subtle, defects reside in the other allele of this locus. Recent work on extrapulmonary small cell cancer, histologically similar to SCLC, revealed loss of chromosome 3p sequences in only 1 out of 5 of these tumours [82]. It is of considerable interest that a deletion at 3p21 detected with pH3H2 has also been observed in renal cancer [83]. However, another study of renal cancers indicates that involvement of 3p12-14 in chromosomal abnormalities is probably more common in this tumour type [84]. Alterations in 3p are not restricted to lung and renal cancers: in ovarian adenocarcinomas clonal abnormalities in the 3p21-25 region have been reported [85]. Molecular cloning of DNA sequences in or very near the 3p21 locus should allow isolation and subsequent characterization of the putative gene in the future. These results must be awaited before the role of 3p21 sequences in the pathogenesis of human lung cancer and the specificity for this type of tumour can be adequately assessed.

Apart from chromosome 3 deletions, other chromosomal alterations have been reported in lung cancer. Cytogenetic studies show that loss of chromosome 13 is a common finding in SCLC cell lines [28, 76], whilst the use of several RFLPs revealed the loss of chromosome 11 sequences in NSCLC [86]. In the latter study loss of heterozygosity was associated with progression of the tumours, and this might, therefore, reflect their genetic instability.

It may be expected that both cytogenetic and molecular studies will disclose additional (micro)deletions, which will aid in the molecular characterization of the pathogenesis of lung cancer.

Conclusions

The study of the mechanisms which underlie the transformation of lung epithelial cells has led to the description of several genetic abnormalities in human lung cancer, involving proto oncogenes as well as specific chromosomal deletions. Although these findings are of great importance for the understanding of the molecular processes which direct cellular transformation, their clinical relevance is limited at present. Amplification of myc family genes appears to be associated with tumour progression but is mainly found in in vitro cultured SCLC cells. In primary tumours of SCLC patients the frequency of this activation is too low to be of use as a possible prognostic factor. The use of potentially valuable information when used as a marker for SCLC tumour progression, is hampered by the fact that no treatment alternatives are presently available. The Kras point mutations specifically found in lung adenocarcinomas might be of use to distinguish these tumours from adenocarcinomas of different origin [87]. The role of this activation for prognosis or classification remains to be investigated. Over-expression of the EGFR, at first assumed to be more or less specific for epidermoid carcinomas, does not seem to be restricted to this NSCLC subtype, and contributes little in the distinction between SCLC and NSCLC. No data are available concerning possible differences in clinical or biological properties of EGFR positive and negative tumours.

Research investigating the potential of anti EGF antibodies for diagnostic imaging and even for the therapy of NSCLC is now in progress. Only the first steps in the elucidation of the molecular basis of lung cancer have been made. The rapid progress in identifying the genes and processes involved in the pathogenesis of this devastating disease is encouraging and exciting for both fundamental scientists and clinicians.

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RÉSUMÉ: Grâce au développement des techniques de biologie moléculaire, la recherche d'altérations génétiques dans les cellules cancéreuses a permis un début de description des transformations moléculaires au niveau cellulaire. Beaucoup de ces modifications génétiques se produisent au niveau des gènes, qui jouent un rôle dans le contrôle de la croissance et du développement cellulaire, les proto-oncogènes. Au cours de la dernière décennie, il est apparu que les familles oncogènes myc et ras sont importantes dans la carcinogénèse des tumeurs pulmonaires humaines. Les oncogènes myc apparaissent habituellement altérés dans les cancers à petites cellules: ces altérations sont en corrélation avec un développement rapide et une progression tumorale. Les mutations du gène K-ras sont spécifiques de l'adénocarcinome, une sous-classe des cancers pulmonaires autres qu'à petites cellules. Les mutations des gènes K-ras ont été décélées chez les adénocarcinomes des grands fumeurs, tandis que dans le groupe sans mutation, les patients étaient des non fumeurs ou des ex-fumeurs de longue date. Les mutations du gène K-ras sont donc étroitement associées à la fumée de tabac. L'oncogène erbB, qui encode le récepteur du facteur de croissance épidermique, est souvent très manifesté dans les carcinomes épidermoïdes. Les rôles d'autres oncogènes, comme raf ou myh, de même que ceux des gènes suppresseurs, doivent encore être investigués, mais pourraient être d'importance essentielle. L'étude des altérations des proto-oncogènes pourrait aider à la sousclassification et au diagnostic du cancer pulmonaire, et pourrait fournir des informations pronostiques utiles au cours des prochaines années.