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Tumour Markers and Oncogenes in Lung Cancer

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INTRODUCTION

LUNG CANCER is the leading cause of cancer death with approximately 90% of affected patients dying within 1 year of diagnosis. Unlike in several other cancer types, the incidence of lung cancer has been steadily growing, possibly due to the strong association of lung cancer and cigarette smoking. On the basis of both clinical behaviour and prognosis, lung cancers can be divided into small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). This division has previously been thought to reflect differences in the cell types from which these tumours arise, but it has been recently suggested that both classes of tumours may have a common progenitor, because a significant fraction of NSCLC tumours (20%) exhibit neuroendocrine properties [1]. In addition, single tumours can contain mixtures of cells including both NSCLC and SCLC types (see below). Finally, transitions from SCLC to NSCLC phenotypes have been reported in several cases [2]. In spite of these results, the distinction between SCLC and NSCLC is clearly useful from the clinical standpoint.

SCLC accounts for approximately 25% of all new cases of lung cancer. Long-term survival or complete remission in SCLC is directly related to the response to cytotoxic therapy; surgical treatment is not a useful alternative. NSCLC includes adenocarcinoma (30%), squamous cell (25%) and large cell (15%) carcinoma. For NSCLC patients, resectability of the primary tumour is the major prognostic factor.

During the last 10 years, medical and surgical intervention has resulted in little change in the 5-year survival rate for lung cancer. Therefore major efforts in research are being directed to identifying relations between specific tumour markers and gene alterations and the clinical behaviour of the tumours. Relevant results from such studies could provide more accurate and useful diagnostic tools, which could be used in a more singular disease assessment and treatment.

TUMOUR MARKERS IN LUNG CANCER

Due to the different therapeutic strategies employed in the treatment of SCLC versus NSCLC, non-invasive diagnostic methods have been more extensively studied in SCLC. Since in general SCLC is highly sensitive to chemotherapeutic agents and has frequently metastasised by the time of clinical presentation, systemic chemotherapy plays a major role in the management of these patients. Therefore reliable tumour markers, in addition to imaging techniques, could yield valuable information in the treatment of these patients.

A wide variety of potential tumour markers has been identified from cell lines established from SCLC tumours (Table 1). These include several enzymes involved in the neuroendocrine amine precursor uptake and decarboxylation (APUD) system and some peptide growth factors and their receptors. Although some of these markers are useful in differentiating between SCLC and NSCLC, several of them have also been identified from a subset

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Table 1. Tumour markers in lung cancer

Biomarkers
NSE
DDC
Chromogranin A and B
CK-BB
CEA
PP-7B2
N-CAM
Cluster 1 Ag
HLA-I
Fucosyl GM-1 ganglioside
Growth factors/receptors
Bombesin/GRP
Bombesin-R
IGF-I
EGF-R
Transferrin
IL-2-R

of NSCLC [3], again suggesting a common origin for these tumours. Markers reported to be rather specific for SCLC cell lines include APUD enzymes L-dopa decarboxylase (DDC), neuron-specific enolase (NSE), the BB isozyme of creatine kinase (CK-BB), gastrin-releasing peptide (GRP or bombesin; [4]), pituitary polypeptide 7B2 [5], chromogranin A and B [6], tetanus toxin labelling of cells [7], the neural cell adhesion molecule (N-CAM; [8, 9]), and the cluster I antigen [10]. In addition, SCLC cell lines have been further divided into lines with classic and variant features. The repertoire of markers expressed by the variant cell lines does not include DDC or bombesin, and these cell lines have several other distinctive features compared with the classic lines [11, 12]. Although specific markers for NSCLC cells have not yet been reported, they can also be distinguished from SCLC cells by the expression of HLA-I, which is almost invariably absent from SCLC cells [13]. Monoclonal antibodies specific for subtypes of NSCLC may soon prove useful [14].

Identification of such a vast array of markers from SCLC cell lines has generated several studies on their applicability in clinical situations. Several reports indicate NSE as the most useful marker for both follow-up [15, 16] and evaluation of prognosis [17]. NSE levels have been measured from tissues by immunocytochemical methods and also from the serum. Using a combination of these assays, up to 90% of SCLC patients show an elevated level of NSE [18]. Several other markers may prove useful in the near future. These include chromogranin A and B [19, 20], GRP [21] or the C-flanking peptide of proGRP [22], soluble interleukin-2 receptors [23], cluster I antigen and other monoclonal antibodies specific for lung cancer subtypes [10], the 7B2 protein [24], CK-BB [25] and the fucosyl GM-1 ganglioside [26].

In addition to markers specific for SCLC, several more general tumour markers have been found useful in clinical follow-up. Although several of these have proven to be too insensitive or unspecific (e.g. calcitonin, ACTH, ADH), carcinoembryonic antigen has been found to be a useful marker in the follow-up of early SCLC relapses [27, 28]. A promising use for several of the markers is in the diagnostics of central nervous system metastases by comparison of the levels of the markers in serum and cerebrospinal fluid [29, 30].

Table 2. Oncogenes in lung cancer

Tumour	Oncogene
SCLC	*myc* family
Adenocarcinoma	K-*ras*
SCLC	PRAD1?
NSCLC	EGF-R
NSCLC	*neu*

ONCOGENES IN LUNG CANCER

Consistent chromosomal abnormalities such as translocations, deletions or abnormally staining chromosomal regions have been identified from a variety of tumours [31, 32]. These abnormalities are often, if not always, associated with the activation of cellular proto-oncogenes [33] or inactivation of tumour suppressor genes [34]. Well known examples include activation of the c-*myc* proto-oncogene in Burkitt's lymphoma due to a translocation juxtaposing it with the active Ig enhancer [35], and inactivation of the Rb tumour suppressor gene due to chromosomal deletions in retinoblastoma [36]. Several different oncogenes and tumour suppressor genes have been implicated in the various forms of lung cancer [37–39]. Some of these are discussed below.

Members of the *ras* oncogene family have been found to be activated frequently in human tumours [40]. Interestingly, activating point mutations in codon 12 of the K-*ras* oncogene were recently found to occur in about one third of adenocarcinomas of the lung, and this mutation was found to correlate with the prognosis of the patients [41].

Elevated expression of the HER2/*neu* receptor oncogene has been linked to a shortened survival in breast and ovarian carcinomas [42]. HER2/*neu* is expressed at high levels in about one third of primary squamous cell and adenocarcinomas of the lung, and this expression is associated with a shortened survival in adenocarcinoma [43].

Abnormalities of the long arm of chromosome 11 are found in various tumour types including translocations in B cell malignancies, and DNA amplifications in squamous cell carcinomas and adenocarcinomas. The chromosomal region involved (band 11q13) contains several oncogenes (*bcl*-1, *int*-2, *hst*, PRAD1), but only PRAD1, a novel cyclin-related gene [44], is consistently overexpressed in all different tumour types affecting this region [45]. This region is also amplified in a subset of squamous cell carcinomas of the lung [46].

Amplification of the three characterised *myc* genes (c-*myc*, N-*myc* and L-*myc*) is commonly detected in SCLC cell lines [11, 47–53], and amplifications of N-*myc* and L-*myc* are consistently also found in primary SCLC tumours (7% and 15%, respectively; [50, 51, 54–60]), while the frequency of c-*myc* amplifications in these tumours is low, and comparable to that found in other lung cancer tumour types [55, 56, 61]. The L-*myc* gene was originally identified as an amplified c-*myc* related gene from SCLC cell lines [62]. Interestingly, L-*myc* has recently also been found to be involved in a gene fusion in two SCLC cell lines [63]. This fusion is due to intrachromosomal rearrangements in chromosome 1 linking regulatory and coding regions from a novel locus named *rlf* to the upstream region of L-*myc* [64]. This results in production of *myc*-L-*myc* fusion mRNAs and protein, which may alter the normal function of L-*myc* (Fig. 1). Similar *myc*-L-*myc* gene fusions have been found from several SCLC cell lines and also from a primary SCLC tumour [54].

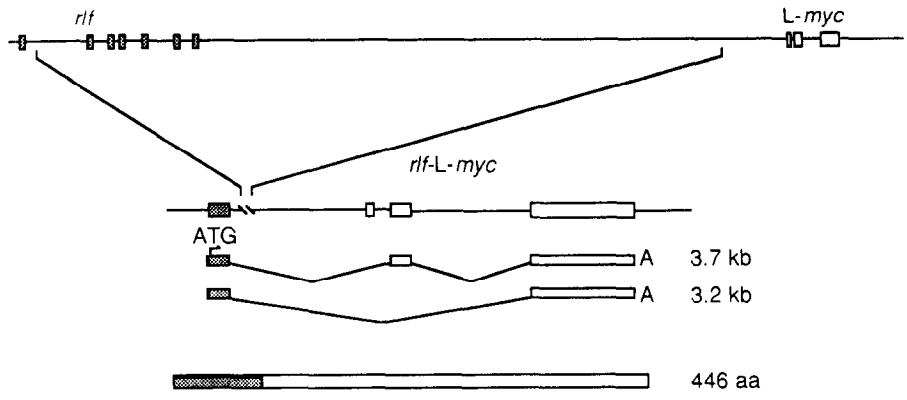

Fig. 1. Intrachromosomal rearrangements in SCLC lead to the production of a *rlf*-L-*myc* fusion protein [63, 64].

All *myc* genes encode nuclear DNA-binding phosphoproteins [65–71], and although the normal functions of the *myc* proteins are not known, a large body of evidence suggests that they may be involved in transcriptional regulation [72–76]. Thus the *rlf*-L-*myc* fusion may alter the normal trans-activating capability of L-*myc*, although protein kinase C induced phosphorylation of the L-*myc* in the trans-activator domain appears to be normal also in the *myc*-L-*myc* fusion protein [54]. We are currently studying the prevalance of the *myc*-L-*myc* gene fusion in SCLC, and also possible functional and/or regulatory effects the *rlf* promoter and coding sequences has on the L-*myc* gene.

Table 3. Tumour suppressor genes in lung cancer

Tumour	Gene	Locus
SCLC	Rb	Chromosome 13q
	p53	Chromosome 17p
	PTP	Chromosome 3p
NSCLC	p53	Chromosome 17p
	nm23?	Chromosome 17q

TUMOUR SUPPRESSOR GENES IN LUNG CANCER

Tumour suppressor genes have been identified either in studies of hereditary cancer (retinoblastoma, Wilms' tumour, neurofibromatosis; reviewed in [34]) or from identification of genes in regions of chromosomes which show loss of heterozygosity (p53, DCC, reviewed in [34]). At least some of these genes have been subsequently found to be inactivated in certain other forms of cancer, including lung cancer.

Mutational inactivation of the retinoblastoma gene on chromosome 13 (13q14) is tightly linked with susceptibility to retinoblastoma, but has also been found in other tumour types including SCLC [77]. Indeed, although initial analysis only revealed abnormalities in 60% of SCLC cell lines, recent reports indicate that the Rb protein product is functionally inactivated in over 95% of SCLC cell lines [77–82] and thus is very likely to play a critical role in the development of this tumour type.

Loss of heterozygosity of markers on the short arm of chromosome 17 occurs with a high frequency in several tumour types including both SCLC and NSCLC in a region which contains the p53 gene [83]. In addition, these tumours exhibit point mutations in the single remaining allele of p53 [84]. Different abnormalities of p53 are found in a high percentage of both lung cancer cell lines and tumours [85–89].

In addition to the described chromosomal abnormalities, SCLC cells almost invariably contain cytogenetically visible deletions [90] or loss of heterozygosity of polymorphic markers [91–93] on the short arm of chromosome 3 (3p14–23). Similar abnormalities are also detected in some NSCLC tumours [92]. Although the putative tumour suppressor gene from this region has not yet been identified, several transcribed sequences in the deleted region have been identified, and appear to be closely linked with the putative suppressor gene [94–96]. Different forms of lung cancer exhibit several additional chromosomal abnormalities [38, 97–100], which are not as consistently found. For some of these, candidate suppressor genes have already been reported (*nm23* [97]).

For several years epidemiological evidence has pointed to the multistep nature of cancer. Recent molecular analyses of lung cancers show multiple genetic lesions in the form of several activated oncogenes and inactivated tumour suppressor genes in a single tumour [38, 89], suggesting cooperative action of these genes in the production of the malignant phenotype [101]. The understanding of these events may help us to unravel the molecular mechanisms of initiation and progression of lung cancer, and provide new approaches to prevention and therapy of these tumours [37]. A multivariant analysis with relevant oncogenes, tumour suppressor genes, and tumour markers may also change our concepts on classification of lung tumours and may provide new powerful tools for the estimation of their prognosis.

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