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Lung Cancer Biology

Desmond N. Carney

INTRODUCTION

IN SPITE of major advances in our understanding of the prognostic factors of patients with lung cancer, little advance has been made in improvement in the overall survival of patients with this disease over the past decade. In the USA it has been anticipated that this year there will be almost 160 000 new cases of lung cancer, and the vast majority of these patients will die from their disease. The 5 year survival rate for all patients with lung cancer remains between 7 and 10%. For patients with non-small cell lung cancer (NSCLC) the only hope for many for prolonged survival is directly related to the resectability of this tumour. In contrast, for patients with small cell lung cancer (SCLC), which accounts for 25% of all new cases of lung cancer, the sensitivity of this type to chemotherapy and/or radiation therapy predicts well for the survival of patients with this disease. However the vast majority of patients with SCLC will have widespread metastatic disease at the time of initial presentation and even with intensive cytotoxic chemotherapy less than 10% of all patients will be cured of their disease. While many factors may influence the overall survival of patients with lung cancer, including performance status and stage of disease, it is now clear that biological factors inherent within the tumour cells themselves may be of clinical importance. These properties include the expression of neuroendocrine markers and the expression of drug or radiation resistant genes or oncogenes coding for more malignant behaviour or specific cytogenetic abnormalities. Here we will review the recently recognised biological properties of lung cancer cells and discuss their application in the management of patients with this disease.

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CELL LINES OF LUNG CANCER

The ability to establish permanent cell lines of both SCLC and NSCLC has greatly enhanced our understanding of the biological properties of these tumour cell types. The development of specifically designed serum-free hormone supplemented medium for the selective growth of both SCLC and NSCLC has provided us with large panels of cell lines to study biological properties [1–6]. SCLC cell lines grow as floating aggregates of tightly to loosely packed cells, while NSCLC grow usually as adherent monolayer cultures. Both cell types form tumours in soft agarose and are tumorigenic in nude mice. Based on their expression of a variety of biochemical markers including L-dopa decarboxylase (DDC), neuron specific enolase (NSE), creatine kinase-BB (CK-BB) and gastrin-releasing peptide (GRP) SCLC cell lines can be subtyped into two major categories, namely, classic cell lines which express high levels of all four of these markers, and variant cell lines which have selective loss in their expression of both DDC and GRP. In addition classic cell lines have a more aggressive growth behaviour *in vitro*, a higher colony forming efficiency in soft agarose and when implanted into athymic nude mice have a shorter latent period to tumour formation.

Recently it has been clearly demonstrated that up to 30% of cell lines or fresh tissue obtained from patients with NSCLC will have features of neuroendocrine differentiation, in particular the expression of high levels of DDC and NSE. Thus it can be observed that both within SCLC and NSCLC two major divisions of cell types can be identified, i.e. those that have evidence of neuroendocrine differentiation and those that lack it. The expression of these neuroendocrine markers may be important

in predicting for patient survival and responses to cytotoxic chemotherapy (see below). The cell lines of SCLC and NSCLC have also provided the basis for our understanding of the molecular genetics of lung cancer, the identification of specific growth factors for these cell types and have provided tools for understanding the patterns of *in vitro* chemosensitivity and drug resistance for lung cancer cells.

THE CLINICAL IMPORTANCE OF THE ESTABLISHMENT OF TUMOUR CELL LINES *IN VITRO*

Recently several investigators have evaluated the prognostic significance of the establishment of a cell line from an individual patient with either SCLC or NSCLC [7, 8]. The establishment of cell lines from patients with SCLC was not statistically significantly correlated with survival time, although the median survival period of patients from whom cell lines could not be established was longer than those from whom cell lines were established *in vitro* [7]. In this study, however, cell lines were only established from patients with extensive stage disease (ED) in whom many other compounding factors may have influenced their overall survival.

In contrast a recent study evaluating the prognostic importance of establishing tumour cell lines *in vitro* from patients with NSCLC has clearly demonstrated that the successful cell line establishment is an important negative prognostic factor for survival and is independent of other non-prognostic factors in patients with NSCLC [8]. In this study 123 consecutive patients with NSCLC from whom viable tumour specimens could be obtained were included. Although specimens were obtained from all patients, cell lines were established from only 25 patients (20% of the total). The median survival of 7 months among patients from whom cell lines were established compared with 18 months in patients from whom cell lines were not established was highly significantly different. It is interesting to note that this prognostic factor was particularly evident among patients from whom tumours were resected for a curative approach, in particular those with early stage disease. Thus these data suggest that in patients with NSCLC the establishment of a cell line is an important new and potentially relevant discriminator of high risk for tumour recurrence and appears to be independent of other prognostic variables. This should be taken into consideration in the selection of patients for adjuvant therapy following potentially curative surgical resection.

THE CLINICAL SIGNIFICANCE OF NEUROENDOCRINE MARKER DIFFERENTIATION IN LUNG CANCER CELLS

The demonstration that both SCLC and NSCLC cells could be differentiated into major categories based on their expression of neuroendocrine markers has led to several studies to evaluate the clinical relevance and significance of the expression of these markers *in vivo*, in particular among patients with NSCLC cells [9–12]. Using a variety of techniques including immunohistochemical staining, electron microscopy, biochemical or immunological assays and/or the use of molecular probes, the incidence of neuroendocrine NSCLC tumours ranges between 10 and 30% of all NSCLCs, in particular among adenocarcinoma cells. In several studies it has been suggested that the clinical behaviour of neuroendocrine NSCLC may be different to non-neuroendocrine NSCLC tumours.

These suggest that neuroendocrine NSCLC tumours have a higher response rate to systemic chemotherapy and that the survival of neuroendocrine marker positive responders was superior to that of nonresponding tumours. The significance of the presence of neuroendocrine markers in NSCLC cells was more important when 2 or more neuroendocrine markers were detected.

While the number of studies carried out has been small it is clear that prospective studies will be required to further validate the importance of neuroendocrine marker differentiation in NSCLC. The presence of these markers may indeed identify a subset of patients with NSCLC whose tumour behaviour may be closely aligned to that of SCLC and who thus may benefit from the use of systemic chemotherapy. Much work has been published on the use of serum markers in the management of patients with SCLC and, indeed, with NSCLC. The results of studies using such markers as NSE, chromogranin-A, CK-BB, etc. have all been published in detail elsewhere. While many of these markers show an excellent correlation with disease extent and stage and reponse to cytotoxic therapy, thus far it would appear that none are either specific or sensitive enough to encourage widespread use in the management of patients with lung cancer. However, prospective studies should be carried out to determine the initial levels of these markers, their rate of fall following the use of cytotoxic therapy and the expression of high levels of serum neuroendocrine markers in patients with this disease.

ANTIGEN EXPRESSION IN LUNG CANCER

In recent years numerous monoclonal antibodies have been generated against lung cancer associated antigens [13–15]. In an attempt to identify monoclonal antibodies that may be of clinical value two recent international workshops on lung cancer antigens have evaluated the usefulness of numerous antibodies raised to lung cancer associated antigens [13, 14]. In these studies antibodies from numerous sources were investigated for their reactivity with both normal and malignant tissue, or panels of cell lines and fresh biopsy specimens. Based on these studies the antibodies submitted to the workshop could be classified into different clusters according to the pattern of reactivity.

The studies demonstrated that many monoclonal antibodies generated react with a similar antigen in tumour cells and that some were highly specific for neuroendocrine cells, including SCLC cells, while others demonstrated cross-reactivity with other epithelial cells. The clustering of these monoclonal antibodies has allowed the definition of many of the epitopes that react with these antibodies. For example, it has been demonstrated that the cluster 1 antigen designated by the workshop on SCLC antigens was the neuro-cell adhesion molecule (NCAMP).

Moreover, the characterisation of the antibodies has permitted the use of a panel of them in a range of investigations in patients with lung cancer. These include the use of monoclonal antibodies (MoAbs) to detect bone marrow metastases; evaluation of antigen expression and its correlation with long-term survival in patients with lung cancer; and the use of MoAbs for tumour-directed therapy in patients with SCLC. In addition there has been a recent interest in the application of MoAbs in the evaluation of sputum immunocytology for the early detection of lung cancer. This technique was associated with a 90% diagnostic accuracy 2 years prior to the eventual diagnosis of cancer using conventional techniques. While this initial report has been on a small number of patients it is clear that the application of MoAbs for tumour localisation and therapy delivery may eventually be more successful if these tumours can be detected at an earlier

phase when the tumour volume is smaller and the likelihood of metastatic disease remote.

MOLECULAR GENETICS OF LUNG CANCER

DNA content analysis of lung cancer tumours has demonstrated that approximately 85% of all tumours will have aneuploid DNA content ranging from hypodiploid to tetraploid. Multiple stem lines are observed in 10–20% of specimens. While studies in SCLC specimens have been inconclusive, several studies have clearly demonstrated that the degree of aneuploidy and the proliferative fraction identified in fresh specimens of NSCLC are of prognostic importance [16–25].

Molecular cytogenetic studies have clearly demonstrated a range of abnormalities present in lung cancer cells involving both dominant and recessive genes. In cell lines of SCLC, members of the *myc* family of oncogenes, namely, c-*myc*, N-*myc* and L-*myc*, may be important in the biology of this tumour type. In many cells of the variant subtype of SCLC up to 76-fold amplification of c-*myc* has been identified. In contrast amplification and/or overexpression of N-*myc* and L-*myc* have been identified, particularly among the classic cell types of SCLC. However, in studies of large numbers of cell lines in fresh biopsy specimens of SCLC, *myc* family oncogene abnormalities are more frequently observed in cell lines compared with fresh biopsy specimens; and in both groups they are usually observed in specimens obtained at relapse from prior induction therapy. Thus these data suggest that the *myc* family of oncogenes are a late event in the biology and pathogenesis of SCLC and might contribute to the more aggressive growth behaviour frequently observed at relapse in patients with this disease. While a variety of other oncogenes have been noted in SCLC, their infrequent occurrence suggests that they have a minimal role to play in the biology of this disease.

Among NSCLC tumours and cell lines the *ras* gene family has been implicated, particularly in adenocarcinoma specimens. In several studies it has been found that activation of the K-*ras* oncogene by a point mutation in codon 12 occurs in about one-third of human lung adendocarcinomas. A recent study has clearly demonstrated that this is of major clinical significance. Among 69 patients with lung adenocarcinoma in whom complete resection of the tumour was possible, 19 of the tumours demonstrated a point mutation of the K-*ras* oncogene. There was no association between this point mutation and the age of the patient, sex or the presence of previous or recurrent neoplasms. The presence of the K-*ras* point mutation was associated with a significantly shorter overall survival and disease free survival compared with the patients in whom no mutation in the K-*ras* oncogene was identified. Thus the presence of K-*ras* point mutations among patients wth primary lung adenocarcinoma who undergo a curative resection defines a subset of patients with a very poor prognosis and short disease free survival. This should now be taken into consideration in defining patients for postoperative adjuvant systemic therapy.

Chromosomal studies in lung cancer specimens have demonstrated a range of structural and numerical cytogenetic abnormalities. Studies comparing tumour and normal tissue DNAs using the restriction fragment length polymorphism probes (RFLPs) have revealed loss of heterozygosity in chromosome regions 3p, 13q and 17p. This allele loss is highly suggestive of the presence of an antioncogene. Among cell types of both SCLC and NSCLC a deletion in the short arm of chromosome 3 has been demonstrated in the majority of specimens, including both fresh specimens and established cell lines. Among SCLC cells this deletion has been identified in both primary and metastatic specimens but it is of note that it has not been identified in extrapulmonary SCLC specimens. Structural abnormalities of the retinoblastoma gene are also present in SCLC cells.

Retinoblastoma mRNA expression is absent in 60% of SCLC cell lines in contrast to 10% of NSCLC cell lines, implying an important role for the retinoblastoma gene in the pathogenesis of SCLC but not of NSCLC. Finally it has been demonstrated that the p53 gene located on chromosome 17p which has many features of an antioncogene is frequently mutated or inactivated in all types of lung cancer. In addition, expression of p53 mRNA in lung cancer cell lines has been found to be very low or absent, as compared to normal lung tissue. These findings strongly implicate p53 as an antioncogene important in the pathogenesis of all cell types of lung cancer.

DRUG RESISTANCE IN LUNG CANCER

Over the past decade much work has been carried out on the *in vitro* chemosensitivity of lung cancer cells [26–28]. For a variety of reasons the use of primary fresh biopsy specimens for predicting *in vivo* chemosensitivity has not been very fruitful. In contrast, studies using permanently established cell lines of lung cancer cells have clearly demonstrated that the pattern of *in vitro* chemosensitivity observed in both early and late cultures is highly predictive of the responsiveness of the patient *in vivo* and thus these should serve as a very useful tool for the screening of potentially new active agents for the treatment of patients with lung cancer.

Cell lines or fresh specimens of lung cancer have been used to better understand mechanisms of drug resistance in lung cancer cells, in particular pleiotropic drug resistance. This latter type of drug resistance is common in lung cancer specimens. In a variety of other human tumour types pleiotropic drug resistance is associated with the expression of P-glycoprotein, a membrane glycoprotein of molecular weight approximately 170 000. However, in studies of lung cancer cells, this glycoprotein does not appear to be important in the majority of cases of drug resistance. With one exception, P-glycoprotein was not expressed above normal background in most tumour types evaluated. These studies demonstrated that there was no correlation between its expression and patterns of *in vitro* chemosensitivity, prior to therapy status and clinical response of the patients to therapy. It was interesting to note, however, that among specimens of NSCLC only those with neuroendocrine properties had high levels of expression of the P-glycoprotein. It is clear therefore that many mechanisms of drug resistance may be important in lung cancer cells. However, the development of cell lines demonstrating both chemosensitivity and drug resistance *in vitro* should provide a useful model for understanding and overcoming these mechanisms of drug resistance.

GROWTH FACTORS IN LUNG CANCER CELLS

The availability of cell lines of lung cancer cells in serum free chemically defined medium has permitted the identification and characterisation of autocrine growth factors from both SCLC and NSCLC cells [29, 30]. Among SCLC cells GRP, insulin-like growth factor-1 (ILGF-1) and transferrin have demonstrated all the characteristics of an autocrine growth factor while in NSCLC cells ILGF-1 has also demonstrated these properties [30]. Understanding their mechanisms of action provides us with alternative therapeutic approaches to the management of patients with this disease. These include the use of monoclonal antibodies to the growth factors and/or their receptors, the use

of antagonists to these growth factors and finally disruption of the signal pathway involved once these growth factors are internalised within the cell. As it is likely that many growth factors are involved in lung cancer, this latter approach may prove to be the most important in therapeutic studies of patients with this disease.

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Haemopoietic Growth Factors: Unravelling the Secrets of Blood Formation

M. H. Bronchud

INTRODUCTION

EVEN BEFORE the Old Testament book of Leviticus was written, mankind had a particular fascination for blood ("for it is the life of all flesh", Leviticus). Most cellular elements of this liquid organ derive from the bone marrow by a process called "haematopoiesis", often abbreviated to "haemopoiesis". The production of blood is the best understood of all the many processes of cellular proliferation and differentiation in the living organism, both in terms of its molecular and its cellular mechanisms. The reasons for the clearer understanding of this fundamental process, when compared to other body tissues, are mainly technical: easy sampling of mature cells from peripheral blood and of progenitor cells from the bone marrow; the production of highly differentiated products by mature blood cells; good morphological markers; and the development over the last three decades of functional assays to measure the number and quality