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THE BIOLOGY OF LUNG CANCER

A review

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Abstract

Advances in the understanding of the biology of lung cancer have progressed rapidly over the last decade. It is clear that considerable heterogeneity exists both in small-cell and non-small-cell lung cancer tumours with common properties being shared by both cell types. To further understand the prognostic and clinical significance of these biological properties it is clear that in future clinical trials of both tumour types these properties should be taken into consideration.

Key words: Lung cancer, biological markers, review.

Lung cancer remains a major health problem throughout the world. Among males in Ireland lung cancer accounts for 35% of all deaths due to cancer, while among females the figure is 19%. In addition, and unlike many of the other common epithelial tumours, the incidence of lung cancer is still on the increase. This year in the United States, there will be approximately 135000 deaths from this disease. While there are four major histological subtypes of lung cancer, because of responses to therapy including radiation therapy and cytotoxic therapy, they are collectively divided into two major subgroups, namely, small-cell lung cancer (SCLC) which accounts for about 25% of all new cases of lung cancer, and non-small-cell lung cancer (NSCLC) which includes squamous cell, adenocarcinoma and large-cell carcinoma. Among patients with SCLC the major hope for cure and long-term survival is directly related to their response to cytotoxic therapy including combination chemotherapy with or without radiation therapy. In contrast, for patients with NSCLC hope for cure and long-term survival is directly related to the resectability of this tumour at the time of presentation. However, for patients with both SCLC and NSCLC, while many of the prognostic features have been identified, including performance status and stage of disease, it has become apparent that considerable heterogeneity exists within individual tumours which may account for the differences in responses to therapy observed. Thus it has become obvious that the biological properties of these tumours may be of significant prognostic importance. These properties include the expression of drug- or radiation-resistant genes or oncogenes coding for more malignant behaviour. In this paper we will review the biological properties of lung cancer cells, both in vivo and in vitro; consider the applications of these properties in the management of patients with lung cancer; and finally consider how the understanding of these biological properties may help us in defining new therapeutic modalities for patients with this tumour type.

Cell lines and biochemical markers of lung cancer cells

Major advances in understanding the biological properties of lung cancer have come from our ability to establish permanent cell lines of both small-cell and non-small-cell cells in vitro. Success in establishing some cell lines derives from the development of defined serum-free hormone-supplemented medium, which can readily support the continuous growth of tumour cells from fresh biopsy specimens obtained from patients with these tumours (Table). Using defined HITES medium for SCLC cells permanent cell lines can be established from 75% of all specimens (1). Such cell lines can be established from a variety of organ sites, such as bone marrow, lung, liver and lymph nodes and once established can be maintained indefinitely in culture. They can be successfully cryopreserved and will form colonies in soft agarose and tumours in athymic nude mice. SCLC cells grow as floating aggregates of tightly packed to loosely packed cells frequently

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demonstrating areas of central necrosis. In contrast NSCLC cells usually grow as attached monolayer cultures (2). NSCLC cells can be more readily established from metastatic lesions than from primary lesions, suggesting that these metastatic deposits may be of a more malignant behaviour type than the primary lesions. When SCLC and NSCLC cells are established as permanent cell lines they retain the morphology, cytology and histological appearances very similar to the original specimens from which they have been established. Stability in culture is usually noted for these lines over prolonged periods. In general, no major biological differences have been observed between cell lines obtained from a variety of different organ sites in patients with SCLC, both from different patients and from cell lines established from individual metastatic lesions in the same patient. The ability to establish such cell lines permits detailed studies of the biological properties of these tumour cells and, in addition, in vitro studies of drug and radiation sensitivity and evaluation of the presence and expression of proto-oncogenes.

A variety of different biochemical markers have been identified which can clearly distinguish SCLC cells from NSCLC cells (3). These include the APUD enzyme L-dopa decarboxylase (DDC), the peptide hormone bombesin (BLI/GRP), the APUD enzyme neuron-specific enolase (NSE) and the BB isozyme of creatine kinase (CK-BB). In general such markers are expressed in high amounts in cell lines of SCLC origin and are rarely observed in those of NSCLC tumours. The identification of such markers in SCLC cells has prompted many studies to evaluate the role of serum markers in patients with SCLC (4). Such studies clearly demonstrate that cell lines of lung cancer can be used to identify biochemical markers which can be of major value in the clinical monitoring of patients with lung cancer.

Detailed studies of a large panel of SCLC cell lines have also demonstrated that considerable heterogeneity does exist among individual cell lines in the expression of these biomarkers. This has permitted the subdivision of SCLC cells into two major classes, namely, classic cell lines which express elevated levels of all four biomarkers, and variant SCLC lines in which there is selective loss of expression of both DDC and BLI/GRP (1). However, and unlike NSCLC lines, the variant lines continue to express elevated levels of both NSE and CK-BB. A variety of other biological properties have also been identified which can clearly distinguish the classic and variant SCLC lines. The variant lines have a more rapid growth behaviour both in vivo and in vitro and a higher colony-forming efficiency than classic cell lines. They are radioresistant and morphologically more closely resemble large-cell undifferentiated carcinoma. Moreover, these variant cell lines have a four-to sixtyfold DNA amplification of the C-myc oncogene and also show increased expression of the gene product (5).

Table

Growth factors for small-cell and non-small-cell lung cancer cell lines and clinical specimens

	Small cell	Non-small cell
Medium designation	HITES	ACL-3
Basal medium	RPMI 1640	RPMI 1640
Attachment factors	–	Collagen/Fibronectin
Hydrocortisone	+	+
Insulin	+	+
Transferrin	+	+
Oestradiol	+	–
Selenium	+	+
EGF	–	+
Sodium pyruvate	–	+
Triiodothyronine	–	+

Although in general the vast majority of NSCLC cultures do not express elevated levels of any of the above markers, it has recently been demonstrated that approximately 20% of cell lines of lung adenocarcinoma express elevated levels of DDC the key APUD enzyme. Thus it has now become clear that heterogeneity in the expression of APUD properties exists both in small-cell and NSCLC cells and that within each tumour type cells exist that express elevated levels of all APUD markers and that tumour cells also exist in which there is selective loss of such markers. While prospecitive studies of the clinical significance of these biological properties of small-cell and NSCLC have not yet been carried out, it is clear that future clinical trials should take these into consideration. Preliminary data do suggest that those patients from whom variant cell lines have been established have a significantly shorter survival time than patients with cell lines of the classic morphology (6). Thus in our future classification of lung cancer, in addition to referring to the histological subtype present, there is a need to classify these tumour cells as endocrine or non-endocrine lung cancer cells based on their expression of APUD properties, as such a classification in clinical trials will determine the prognostic significance, if any, of these properties in lung cancer cells.

Clinical application of tumour markers in lung cancer

Many investigators have previously reported on the use of a range of biomarkers including calcitonin, ACTH, ADH, etc. in both the diagnosis of patients with lung cancer and in monitoring their disease progression and response to therapy. However, many of these markers are neither sensitive enough nor specific enough to warrant their widespread use in the management of patients with lung cancer. In recent years, however, considerable interest has been generated in screening patients with lung cancer for biomarkers detected with high frequency in cell lines of these tumour types including NSE and CK-BB (7–9).

Detailed studies of large numbers of patients with SCLC have demonstrated that serum NSE is elevated in approximately 75% of all patients including the majority of patients with extensive stage disease and approximately 50% with limited stage disease. Serum levels of NSE fall in response to therapy and rise again following relapse (8). In some studies it has been demonstrated that serum levels of NSE become elevated for up to 12 weeks prior to the clinical detection of relapse. Thus sequential measurements of serum NSE may be of value in the early detection of relapse of patients with SCLC. However, it only becomes important when second-line salvage chemotherapy becomes effective in patients who have relapsed from primary cytotoxic chemotherapy in SCLC. It is interesting to note that up to 15% of patients with NSCLC have elevated serum NSE, suggesting that their tumour may be either a mixed tumour (small-cell/adenocarcinoma) or an 'endocrine' NSCLC. Preliminary data suggest that such patients will respond to cytotoxic therapy and consideration might be given to treating these people with SCLC therapeutic regimens.

Studies of serum CK-BB have also been carried out on patients with SCLC. Although elevated levels were detected in only 28% of all patients, the majority of whom had extensive stage disease, initial elevated levels of serum CK-BB appeared to be of prognostic importance in that the median survival of patients with an elevated level was 5 months in contrast to the 12 months of those patients whose initial serum CK-BB was within the normal range. Further studies are required to clarify these data (10). Recently, other investigators have reported on the measurements of serum chromogranin A in patients with SCLC. Chromogranin A is a 68000 dalton protein which is found in neurosecretory granules of normal and malignant APUD cells including SCLC and thus would appear to be a marker of value in patients with this disease. Indeed among a large number of patients elevated levels in the serum were noted in 53% of patients with limited stage disease and 72% with extensive stage disease and medium levels were significantly greater in those with extensive disease (10).

Finally, recent data have again suggested that serum measurements of CEA may also be of value in patients with lung cancer (11). While non-specific, it would appear that patients wiht SCLC whose CEA is significantly elevated at the time of diagnosis have a significantly shorter survival than those patients with a low or normal CEA level. In addition, and after adjustment for performance status and disease extent, the patients with a pretreatment normal CEA had a significantly better response and survival than those with an elevated level. These data are very similar to that reported for CK-BB determinations in SCLC patients.

Perhaps the greatest clinical value of these biomarkers may be in the early detection of CNS metastases in patients, particularly those with SCLC. Several studies in which biomarkers were measured in the cerebrospinal fluid of patients suspected of having CNS metastases, elevated levels of ACTH, ADH, calcitonin, CK-BB and BLI were noted in up to 85% of patients who had confirmed metastatic disease. Of interest, however, was the observation of differential expression of these biomarkers depending upon the site of CNS metastases. The frequency of elevated CSF calcitonin was the same for patients with either parenchymal or meningeal metastases. In contrast, selected elevations of CSF CK-BB and BLI were noted only in patients with meningeal metastases. Thus CSF measurements of these markers may not only be of value in the early detection of intracranial disease, but also in distinguishing meningeal from parenchymal cranial metastases (4, 12, 13).

It should also be noted that while serum and CSF determination of these biomarkers may be of value in managing patients with lung cancer, the detection of such biomarkers in fresh biopsy specimens by immunohistochemical technique may ultimately be of significant importance for classification of lung cancer cells into endocrine and non-endocrine tumour specimens based on their expression of these biomarkers and thus be of therapeutic importance. At the present time because of the non-specificity of some of the assays these studies appear unreliable. With improvement in antibodies and techniques, however, it is clear that they will become of major importance.

Antigen expression in lung cancer cells

Numerous monoclonal antibodies have been generated against lung-cancer-associated antigens. However, because of their lack of specificity and because of problems associated with the biological heterogeneity in antigen expression their use in the diagnosis, staging and treatment of lung cancer patients has been extremely limited. More recently, however, Souhami et al. (14) have reported on the results of an international workshop on lung cancer antigens evaluating the usefulness of numerous monoclonal antibodies which have been raised against lung-cancer-associated antigens. Results using both fresh biopsy specimens and established cell lines have demonstrated that many monoclonal antibodies available cross-react with the same epitope in lung cancer cells. However, based on studies of approximately 54 monoclonal antibodies the reaction patterns of such antibodies could be subdivided into six clusters. These studies also demonstrated that some antibodies were highly specific for neuroendocrine cells including SCLC, while others demonstrated cross-reactivity with a variety of epithelial cells. Further studies are underway, however, to evaluate the prognostic value of specific antigen expression by tumour cells in relationship to response to therapy and survival. It has also been demonstrated recently that a variety of antigens normally expressed in haematopoietic cells can be expressed in SCLC cells (15–17). In addition, expres-

sion of such antigens can be modulated by a variety of biologics including interferons. The reason why SCLC cells and haematopoietic cells including NK cells share common antigens is unclear but may relate to a specific aspect of SCLC biology including its high propensity to early dissemination and spread.

Several studies have also demonstrated a differential expression of the Class I major histocompatibility complex antigens between non-small-cell and SCLC cells. These antigens are either not expressed or expressed at very low levels in SCLC cells (18). Studies have shown that the genes for these antigens are intact at the DNA level but in most cases HLA and β_2-microglobulin mRNA are not expressed or expressed at very low levels. Incubation of SCLC cells with interferon leads to the development of detectable amounts of these antigens on cell surface both in vitro and in vivo. Thus it is clear that modulation of the expression of antigens in lung cancer cells can take place in vivo and agents which may modulate or increase this expression may ultimately be of therapeutic benefit. In spite of their relative lack of sensitivity and specificity several studies have been carried out utilizing or evaluating the role of monoclonal antibodies in the detection of metastatic SCLC cells in bone marrow aspirate biopsy specimens (19). In general, among newly diagnosed patients with SCLC, tumour cells will be identified by routine pathological techniques in the bone marrow in 20–25% of all patients. In some studies using monoclonal antibodies SCLC cells were identified in 75% of specimens including many specimens considered pathologically negative for tumour cells. Clearly the detection of tumour cells in bone marrows pathologically negative by standard criteria has significant implications for autologous bone marrow transplantation when it is being considered as part of the therapeutic strategy for patients with this disease. In addition to improve detection, several monoclonal antibodies have been developed which are cytotoxic to tumour cells. Such antibodies offer the potential for the selective in vivo treatment of SCLC patients.

Growth factors for lung cancer cells

It has recently become clear that many cancer cells have the ability to produce, secrete and respond to their own growth factors which thus may account for their ability to regulate their own growth rate. It is also likely that many oncogenes function in malignant cells by altering the production or receptor binding of such autocrine growth factors.

Recent studies have identified several growth factors which are synthesized and secreted by lung cancer cell lines (20–22). The tetradeca peptide bombesin and its related peptide GRP are potent mitogens for both normal lung epithelial cells and SCLC. As it has been previously demonstrated that SCLC cells have high affinity binding receptors for bombesin/GRP, these data coupled with the known mitogenic effect of bombesin/GRP on SCLC suggest that this peptide may function as an important autocrine growth factor for this tumour cell. This hypothesis has been supported by the demonstration that a monoclonal antibody 211, which binds to the C-terminal portion of bombesin, inhibits the growth of SCLC both in vivo and in vitro. Other putative growth factors for SCLC include insulin-like growth factor I (IGFI) which is mitogenic for these cells and the peptide physalaemin which is known to inhibit the growth of SCLC in vitro. Studies of NSCLC have demonstrated these tumours to have increased expression of EGF receptor, suggesting that the epidermal growth factor itself may be a potent mitogen for these tumours. Other studies of NSCLC cell lines have shown that multiple growth factors (TGFα; TGFβ etc.) can be produced and secreted by a single cell line, suggesting that growth factors for these tumour cells may be both multiple and heterogeneous in their activities. The identification of growth factors for lung cancer cells opens new avenues for the treatment of these tumours.

Oncogene expression in lung cancer cells

Numerous studies have been reported on detailed cytogenetic studies in SCLC. These data have confirmed that a specific deletion in chromosome 3 is present in almost every sample of fresh specimens of SCLC in addition to permanently established cell lines of this tumour type. Investigations have shown that the SCLC tumour cells are homozygous for the 3p locus. Whether the 3p deletion is specific for SCLC remains to be determined (23) as such a deletion, however, has also been identified in some cell lines of NSCLC lineage.

Studies of oncogene expression have also demonstrated in a majority of SCLC cell lines amplification of one more of the myc family of oncogenes (C-myc, N-myc and L-myc). Amplification of C-myc is seen most frequently in variant cell lines. In cell lines derived from previously treated patients amplification was present in 44%. In contrast, only 10% of cell lines derived from untreated patients were amplified. In addition, studies of 24 fresh biopsy specimens of SCLC revealed amplification in only 3 (12%) of these. Thus these data suggest that the increased expression and amplification of these oncogenes may in some cases be related to tissue culture techniques. In addition, as changes are most frequently observed in relapsed patients, they suggest a role for these oncogenes in the progression of SCLC.

A common stem cell for lung cancer tumours

While historically it has been widely accepted that cell lines of SCLC origin are of different lineage than those of NSCLC tumours, recent data do suggest that a common stem cell indeed may be involved giving rise to all tumour types. These data include: 1) the frequent recognition of

mixed histologies in biopsies of patients wiht lung cancer; 2) the demonstration that the predominant intermediate filament type proteins in both SCLC and NSCLC are cytokeratins; and 3) the recognition that approximately 20% of adenocarcinomas of the lung express the range of APUD biomarkers more frequently associated with SCLC. In addition, antigenic expression and its pattern is frequently similar between both tumour types. This sharing of properties by both SCLC and NSCLC cells suggests a common stem cell origin. As biological studies have demonstrated that significant differences exist in both radiation and chemosensitivity of cell lines that express or lack endocrine properties, future studies should consider that lung carcinomas in addition to their basic histological parameter should be further classified according to their expression of a range of biological properties as noted above.

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