

Review article

Studying lung cancer in the laboratory: 3—Use of cell lines to investigate the biology of lung cancer

Our understanding of the origins and biology of lung cancer has been enhanced in recent years by the development of laboratory systems that permit the continued growth and characterisation of tumour cells taken from the patient. Most of this work has been performed by establishing small cell lung cancer cell lines in tissue culture.

Evaluation of biological products

Ectopic production of polypeptide and steroid hormones has been well documented in patients with small cell lung cancer^{1,2} and in small cell lung cancer xenografts.³⁻⁵ The use of defined serum free media has allowed easy evaluation of the production of these substances by small cell lung cancer cell lines.^{6,7} In one study of 13 different lines,⁷ appreciable amounts of 14 different hormones were found, with up to 10 different hormones produced by any one cell line. The exact meaning of hormone production by these tumours in patients is unclear. Some studies have shown a positive correlation between serum concentrations and extent of disease or clinical response,^{8,9} while others have found no such effect.^{2,5} No attempt has been made to clarify this problem by studying hormone production by tumour cells in vitro.

Recently much interest has been shown in the production of three other biological "markers" by small cell lung cancer cell lines. Neurone specific enolase, the neuronal form of the glycolytic enzyme enolase, has been found in neuroendocrine (APUD) tumours and is secreted by small cell lung cancer cell lines.^{10,11} The BB isoenzyme of creatine kinase (CK-BB) has also been detected in large amounts in small cell lung cancer cultures¹² but, like neurone specific enolase, is not found in non-small cell lung cancer cell lines (table). A good correlation between serum concentrations and both disease extent and response to treatment has been demonstrated for both these

markers in patients with small cell lung cancer,^{1,12} and one report suggests that CK-BB may be useful as a diagnostic aid in cases of occult tumour.¹³

The neuropeptide bombesin, which occurs physiologically at low concentrations in many tissues, has been shown to be produced at high concentrations by small cell lung cancer cell lines.^{14,15} Specific membrane receptors for bombesin and related peptides have been identified on small cell lung cancer cells¹⁶ and there is good evidence that these substances can stimulate proliferation of small cell lung cancer cells, thereby acting as autocrine growth factors. Further proof of this function came from a study where a monoclonal antibody to bombesin was used to block its binding to receptors on small cell lung cancer cells.¹⁷ Inhibition of clonal growth in vitro and regression of xenografts in vivo were seen. This novel approach to manipulating the growth of small cell lung cancer cells may offer a more rational alternative to cytotoxic drugs in treating patients with small cell lung cancer. Clinical studies evaluating the use of bombesin antagonists or antireceptor antibodies in small cell lung cancer are eagerly awaited.

Cytogenetic studies

Continuous cell lines provide good specimens for conducting tumour cytogenetic analyses. Abnormalities in chromosome numbers and frequent structural aberrations are seen in all small cell lung cancer cell lines.^{6,18-20} One study¹⁹ reported a consistent deletion of the whole or a portion of the short arm of chromosome 3. This abnormality appears to be an acquired somatic cell defect specific to small cell lung cancer. The exact significance of its occurrence is unclear as little is known of the genes present in the 3p region and not all studies of small cell lung cancer have confirmed the presence of this defect.¹⁸ Similar uncertainty regarding clinical relevance also exists over the reports of amplification and expression of certain oncogenes by lung cancer cell lines.²¹⁻²³

Small cell lung cancer has many heterogeneous features with respect to morphology, functional ability, and DNA content.²⁴ Although its cell lines have been shown to have a DNA content similar to

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Property	Small cell lung cancer classic	Small cell lung cancer variant	Non-small cell lung cancer
<i>Morphology</i>			
Culture appearance	Tight aggregates	Loose aggregates	Adherent growth
Nucleoli	Inconspicuous	Prominent	Prominent
Nuclear : cytoplasmic ratio	High	Medium	Variable
Dense core granules	Present	Absent or rare	Absent
<i>Growth properties</i>			
Doubling time (mean (SD))	72 (12) hours	33 (2) hours	About 50 hours
Cloning efficiency	1–5%	10–30%	0·5–40%
<i>Biochemical markers</i>			
L-dopa decarboxylase	Increased	Absent	Absent
Bombesin	Increased	Absent	Absent
Neurone specific enolase	Increased	Increased	Absent or low
Creatine kinase	Increased	Increased	Absent or low
Hormone production	Frequent	Seldom	Seldom
Radiation sensitivity	Sensitive	Resistant	Resistant
C-myc oncogene	Not amplified	Often amplified	Occasionally amplified

that of their original tumour,²⁵ there is now evidence that genetic instability may occur during prolonged growth and repeated passage in vitro.²⁶ Does this property invalidate the use of continuous cell lines to study the biology of the disease?

Major alterations in tumour morphology, biochemistry, chemosensitivity, antigen expression, and DNA content have been observed in patients with small cell lung cancer.²⁵ This suggests that tumour evolution in vivo is a dynamic process, perhaps caused by interactions between clonal subpopulations within any one lesion. Although the selection pressures that may cause these changes in patients (for example, host response, vascularity, treatment) may be different from those encountered by cells in vitro (for example, nutritional requirements, presence of growth and inhibitory factors), the fact that cell lines show genetic instability with time would not appear to invalidate them as experimental models.

The development of drug resistance in patients with small cell lung cancer, often after a dramatic initial response, is probably due in part to heterogeneity in the sensitivity of different subpopulations of cells within a small cell lung cancer tumour. Studying the genetic aspects of cell lines may provide important clues about the cause of this major clinical problem.

Classic and variant cell lines

After the comprehensive characterisation of small cell lung cancer cell lines by Minna and his colleagues,²⁷ two distinct subgroups have emerged. Initially the classification of lines into “classic” and “variant” types was based on their expression of the key APUD enzyme L-dopa decarboxylase, which was absent in variants. Further studies showed important differences in morphology, growth properties, biochemistry,²⁷ and radiosensitivity²⁸ between the two

groups (table). The classic group accounts for around 70% of small cell lung cancer cell lines, variant types comprising the remainder, though there is a certain degree of overlap between the groups. The variants have been further divided into two subclasses with altered biochemical and morphological characteristics.²⁹

The clinical importance of these two classes of cell lines remains uncertain. In general terms the variant group shows more “aggressive” in vitro behaviour than the classic group, with shorter doubling times, higher cloning efficiency, increased oncogene amplification, and decreased sensitivity to radiation. Morphologically, cells of the variant group often resemble large cell tumours and most variant lines have been established from patients with a mixed small cell and large cell morphology. Clinically these patients may have a poorer prognosis than those with the histological appearances of “pure” small cell lung cancer.^{30 31} Perhaps therefore the variants merely represent the in vitro model of this mixed cell subtype of small cell lung cancer.

The fact that the variant cell lines have been seen to evolve from classic cell lines over the passage of time, with loss of APUD properties and an increase in growth rate,^{27 29} adds weight to the idea that different histological subtypes of lung cancer may be seen within the same tumour.^{32 33} It may also support the idea that all types of human lung cancer are derived from a common endodermal “stem cell” origin,³⁴ although this evolution from classic to variant types would appear to represent a process of dedifferentiation.

Conclusions

Advances in tissue culture techniques have made it possible to establish and grow human lung cancer as

continuous cell lines and as xenografts in rodents. Important characteristics of the original tumour such as morphology, chromosome number, and functional activity are retained by tumour cells in these models despite repeated passage over many years.

Over the past five years a wealth of information on the biology, biochemistry, and cytogenetics of human lung cancer has been generated by studies based on laboratory models. Much is now known about the specific growth requirements of lung cancer cells growing in vitro and the factors that regulate tumour cell proliferation. It is hoped that these advances in understanding can be translated into a form that would benefit patients with lung cancer. The development of newer, more effective treatment for patients with widespread disease is by far the most pressing need.

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