Review Article

Studying lung cancer in the laboratory: 1—Development of model systems

Lung cancer is a major health problem in the Western world. It is the leading cause of death from malignant disease in men over the age of 35 and the second leading cause of cancer mortality in women as a whole.\(^1\) The incidence of the disease continues to increase in the female population at an alarming rate. Modern medicine has made little impact on the natural history of the condition, surgery still offering the best chance of cure to a small minority of patients with resectable tumours. Intensive combination chemotherapy has improved life expectancy in some patients with small cell lung cancer, but long term survival in this group is rare.

Although few important advances in the clinical management of the condition have been seen in the past decade, there has been an explosion of information on the basic cellular and molecular biology of the disease. This research has been facilitated by technological developments that have made it possible to grow human lung cancers for prolonged periods in the laboratory. In vitro and in vivo models have allowed both the study of the biological characteristics of the disease and assessment of the chemosensitivity of individual tumours to be performed in the laboratory.

Most of this progress has originated from cancer research establishments engaged in basic laboratory research. Few reports of this work have appeared in respiratory medicine publications, and it is disappointing that more of the respiratory physicians and thoracic surgeons who spend much of their time treating patients with lung cancer are not working in this exciting area of research. This article, the first of three on the study of lung cancer in the laboratory, reviews the methods by which cancer cells can be grown in the laboratory to provide models of human lung cancer.

**Growth of lung cancer in vitro**

Tumour specimens can now be taken from patients and their viability maintained in vitro by means of various tissue culture techniques. Malignant cells will readily form colonies in agar\(^2\) but this method does not allow prolonged growth. In recent years many workers have succeeded in establishing human lung cancer cells in continuous culture by the use of specific media to support cell growth and proliferation. These cell lines have proved invaluable in providing large quantities of malignant cells to aid the study of the biology, biochemistry, immunology, cytogenetics, and chemosensitivity of lung cancer.

**Culturing of clinical specimens in agar**

Successful growth of colonies of lung cancer cells in agar has been reported in specimens obtained from various sites.\(^3-11\) The overall success rate is 50–80% irrespective of histological type. Direct tumour biopsy specimens taken at thoracotomy or necropsy\(^3-6\) provide large amounts of tissue, although the viability and cloning efficiency of these cells may be reduced by both mechanical and enzymatic disaggregation.\(^6,7\) Metastatic deposits in lymph nodes, liver, bone marrow, and the pleura provide accessible and more easily processed specimens, and colonies of lung cancer cells have been successfully cultured from these sites.\(^8-10\)

The diagnostic yield of bronchoscopic washings has been shown to be enhanced when attempts are made to culture cells in vitro. In a study of 39 specimens from 30 patients, Von Hoff and colleagues\(^11\) showed that in three cases lung cancer cell colonies grew from specimens that were negative for malignant cells on routine cytological screening. Bone marrow culture of specimens from patients with small cell tumours showed the same phenomenon. The practical value of using such an assay to screen clinical specimens is, however, debatable as a considerable proportion of the samples from bronchoscopic washings contain bacterial contaminants that will also grow readily in agar.\(^11\) Perhaps the cloning assay could be of value in screening the marrow of patients with small cell lung cancer for occult tumour cells, especially in the case of patients undergoing autologous bone marrow transplantation during intensive combination chemotherapy.

**Growth of continuous cell lines**

For many years investigators have attempted to establish human lung cancers in continuous cell culture. Early studies using slices of tumour tissue were...
unsuccessful, with overgrowth of non-malignant stromal cells preventing the growth of cancer cells. The use of better techniques has allowed successful culture in 10–20% of small cell lung cancer specimens grown in basal media supplemented with fetal bovine serum. The choice of culture medium is now seen to be vital to the successful establishment of cell lines. Studies from the National Cancer Institute at Bethesda, Maryland, where much of the work in this area has been pioneered, have shown that the success rate of growing cultures from biopsy specimens from patients with small cell lung cancer could be increased to 50% by the addition of “conditioned” media taken from already established cultures. This suggested that actively growing cells produced “growth factors” which stimulated their own proliferation and that these factors may not be present in fetal bovine serum. Earlier work had shown that it was possible to maintain cell growth in a medium supplemented with hormones rather than serum, and this led Carney’s group to evaluate the use of serum free media supplemented with various growth factors and hormones in their small cell lung cancer lines. They produced a serum free medium (RPMI-1640 basal medium supplemented with hydrocortisone, insulin, transferrin, 17-β-oestradiol, and selenium—“Hites” medium) that was highly selective for small cell lung cancer cell growth, causing early and rapid proliferation of cells. With the use of such media a large number of small cell lung cancer cell lines have been developed in many laboratories. Non-small cell lung cancer has always proved more difficult to establish in continuous culture than small cell lung cancer, with only sporadic reports of success before 1980.

With the use of defined media, however, more non-small cell lung cancer cell lines are now becoming established. Small cell lung cancer cell lines usually grow as floating aggregates of cells (fig 1), with a fairly long doubling time in culture of about 72 hours (range 36 hours to 3–4 weeks). Non-small cell lung cancer lines grow predominantly as adherent monolayers (fig 2a), although cultures of floating aggregates of cells are occasionally seen. Their doubling times range from 18 to 130 hours (mean 40–50 hours), depending on the medium used for culture. All lung cancer cell lines will form colonies when grown in agar (fig 2b) and when inoculated into immunodeficient mice will produce xenografts with the histological characteristics of the original tumour (fig 2c).

Growth of lung cancer in vivo

A major problem of using in vitro systems to study the biology and chemosensitivity of a solid human tumour lies in the fact that to prepare cultures and perform assays the tumour must first be disaggregated to produce a suspension of single cells. This process, whether performed mechanically or by enzymes, is itself known to have an appreciable effect on the metabolic and cyclic processes of the cell but, perhaps more importantly, the organoid structure and physiological heterogeneity of the solid tumour is lost. In an attempt to preserve this physiological milieu intact many workers have attempted to heterotransplant human lung tumours into various animal hosts.

Types of immunodeficient host

Early workers attempted to grow human cancers in “immune privileged” sites (for example, eye and brain) in normal animals, with limited success. Short term growth of tumours was then obtained in animals immunosuppressed by corticosteroids, irradiation, antilymphocyte serum and cytotoxic drugs. It was then reported that prolonged immunosuppression could be achieved in mice if thymectomy in the neonatal period was combined with total body irradiation in adult life. This type of host can be housed under conventional conditions and is capable of supporting the serial growth of human lung cancers over many years.

At present the most commonly used in vivo model for growing lung cancers in the laboratory is the athymic or “nude” mouse. Immunological studies of these hairless mutants show absence of T lymphocytes in thymus dependent areas of the spleen, a normal complement of B cells, high levels of natural killer (NK) cells, and increased macrophage activity. The main disadvantages of these animals are the strict
Fig 2  Adenocarcinoma cell line (A549): (a) as a confluent adherent monolayer on plastic (no stain); (b) two colonies in agar (no stain); (c) as a xenograft maintained in an immunodeficient mouse (haematoxylin and eosin).
isolation procedures and specialised husbandry techniques required to ensure a healthy colony. Their improved receptivity for human tumours, however, outweighs these problems.

CHARACTERISTICS OF LUNG CANCER XENOGRAFTS

Many reports have described the establishment and maintenance of lung cancer xenograft lines in nude mice,\textsuperscript{32-35} thymectomised irradiated mice,\textsuperscript{29,36} and other animal models.\textsuperscript{24} Successful growth from clinical specimens has been achieved in 44–78% of tumours from reported series. There is little difference in growth between the various histological types of lung cancer or between primary and metastatic tumour. Surprisingly, small cell tumours usually prove the most difficult to establish.

Since the size of subcutaneously implanted xenografts can be easily measured with callipers, much is known about their growth rate. Most workers report doubling times in established xenografts of between six and 29 days.\textsuperscript{29,37} The exact reason for the wide range of doubling times is unclear. Growth rates vary with histological type but, rather surprisingly, small cell xenografts do not have the shortest doubling time.\textsuperscript{29,33,37}

It is essential for human lung cancer xenografts to maintain the characteristics of the original donor tumour if they are to provide a relevant laboratory model for the study of human lung cancer. Several workers have verified that serially passed tumours retain the correct chromosome complement and histological features similar to those of their donor tumour.\textsuperscript{29,34,36,37} The persistence of functional activity, such as ectopic hormone production by small cell lung cancer, has also been found when these tumours are grown in mice. Shimosato \textit{et al}\textsuperscript{34} grew a small cell lung cancer tumour from a patient with inappropriate secretion of antidiuretic hormone and showed evidence of the production of adrenocorticotropic hormone (ACTH) and \(\beta\) melanocyte stimulating hormone 10 months later in the xenograft. In a larger study\textsuperscript{38} ACTH was found in 78% and calcitonin and \(\beta\) human chorionic gonadotrophin in 13% of small cell lung cancer xenografts. Tumour related antigens such as the carcinoembryonic antigen have been found in an appreciable number of transplanted bronchial tumours.\textsuperscript{35,38}

Although human tumour xenografts closely mimic their parent tumour in terms of karyotype, morphology, functional activity, and chemosensitivity,\textsuperscript{37} two important discrepancies exist and are poorly understood. Firstly, it is not clear why human malignant tumours frequently cause substantial weight loss and cachexia in patients but not when transplanted into experimental animals, even when xenografts are allowed to grow to large sizes. Cachexia is now thought to be mediated in patients by a protein named cachectin (tumour necrosis factor) produced by host macrophages.\textsuperscript{39} Considerable variation is observed between different species of mammals, both in the sensitivity to administered cachectin and in the amounts of cachectin produced endogenously after challenge with endotoxin. The production and pathophysiological effects of this factor in immunocompromised rodents bearing human tumours have not been extensively studied.

The other major discrepancy lies in the fact that human malignant tumours frequently appear to lose their ability to metastasise when transplanted into experimental animals. The reason for this is unclear, although it has been suggested that the site and method of implantation may be important.\textsuperscript{40,41} The degree of immunosuppression of the host also appears to be critical in the development of metastases\textsuperscript{40;}, different strains of nude mice are known to show different levels of immunosuppression, the number of NK cells perhaps having a vital role.\textsuperscript{42} To overcome this problem interest is now being shown in the development of animal models of lung cancer with metastatic potential.\textsuperscript{22}

These new developments should provide relevant laboratory tools to help us to understand the biology of lung cancer and hence to allow more effective treatments to be designed in the future.

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