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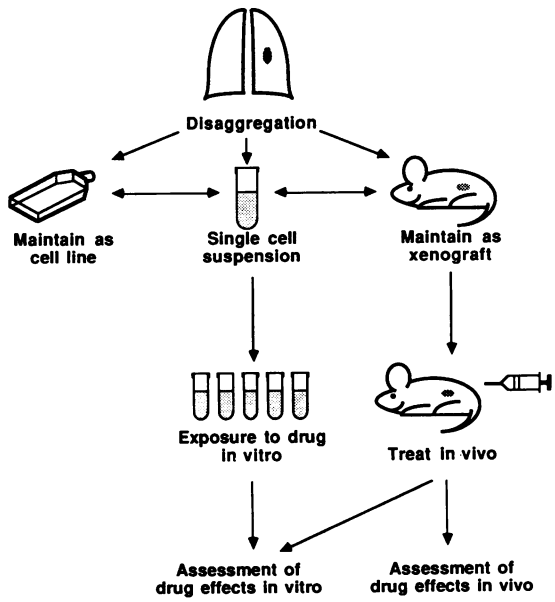
Review article

Studying lung cancer in the laboratory—2: Chemosensitivity testing

The success of in vitro testing of the sensitivity of bacteria to antibiotics stimulated interest in the possibility of developing a similar system for cancer cells, so that the sensitivity of human tumours to cytotoxic agents could be rapidly assessed in the laboratory. There would be two important uses for such a test. Firstly, it would be invaluable for screening new compounds and drug regimens for potential use in treating patients with cancer. Secondly, with pretreatment testing of a patient's tumour against a wide range of agents it might be possible to tailor a patient's treatment individually. This would diminish the use of inactive drugs and their associated toxicity.

Such a test would ideally be simple, cheap, rapid, easily standardised, capable of allowing for various methods of drug action, and able to predict the effect that would be seen in the patient with reasonable accuracy. At present no single technique has satisfied these criteria despite the development of many different in vitro assays and animal model systems (figure).



Methods of assessing anticancer agents in the laboratory.

Short term in vitro assay systems

Most in vitro test systems require the preparation of suspensions of tumour cells, which are then exposed to a fixed concentration of cytotoxic agent. Drug induced cell damage is then assessed according to several different criteria, thus allowing the activity of different drugs on one tumour cell type to be compared.

Several technical problems are encountered at each of these steps. Firstly, the process of disaggregation of a solid tumour to a cell suspension has profound effects on the viability and chemosensitivity of the cells.[1–4] Attempts have been made to overcome this problem by growing tumour cells in culture as spheroids.[5] These spherical aggregates of cells provide a system intermediate in complexity between "solid" tumours and monolayer cultures and represent an attempt to simulate the effect of variation in drug penetration and metabolic gradients that are seen with tumours in vivo. Drug testing of human small cell lung carcinoma cell lines has been reported with this method.[6]

When tumour cells are exposed to an anticancer agent various changes in cellular function are observed. The short term in vitro assay systems that have evolved over the past 25 years constitute several different methods of evaluating these changes. The advantages and disadvantages of each method are summarised in the table.

The earliest assessment of the effects of cytotoxic agents in vitro depended on a crude subjective evaluation of morphological cell damage on the basis of explant cultures.[7] The ability of viable cells to exclude supravital stains has been used in assay systems for many years.[8] Cole has recently described the results of a colorimetric assay in numerous lung cancer cell

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Advantages and disadvantages of the various laboratory systems for drug testing

Test system	*Advantages*	*Disadvantages*
Assessment of cell morphology	Successful growth in culture not required. May be performed on all tumour types.	Large subjective element in assessing cell damage. Delayed effects will not be detected. Semiquantitative at best.
Cell viability and metabolism	Fast and simple. Successful growth in culture not required. May be performed on all tumour types.	No distinction between malignant and non-malignant cells. No distinction between clonogenic and non-clonogenic cells. Timing of testing is critical.
Radioactive nucleotide incorporation	Simple and rapid. May be performed on tumour slices. May be performed on all tumour types.	Nucleotide may be toxic to cells. Some drugs cannot be tested—for example, vinca alkaloids. Changes in nucleotide pools may be misleading.
Clonogenic assays	Test is performed on cells responsible for tumour growth (stem cells). Fibroblast overgrowth prevented. Drugs can act for several cell cycles.	Not all tumours can be tested. Not all drugs can be tested. Tumours must be dispersed into single cells. Time consuming and costly.
Xenograft systems	Drugs requiring in vivo metabolic activation can be tested. Drugs can act for several cycles. Testing of drug combinations is possible. Testing performed in "physiological" environment.	Not all tumours will grow in animals. Altered growth kinetics of xenografts? High cost of animals. Results available in 2–3 months.

lines.[9] In this system a tetrazolium salt (MTT) is converted to a coloured formazan product by enzymes active only in viable cells. The colour reaction can be measured rapidly, allowing the simultaneous testing of multiple cell lines with numerous drugs. The clinical validity of this assay system has not been established.

Measuring the incorporation of radiolabelled nucleosides (for example, uridine, thymidine) into tumour cell preparations during in vitro drug exposure is widely used as a chemosensitivity assay. Bronchial tumours have been extensively studied with this method by Volm and colleagues,[10 11] who showed a good correlation between the sensitivity of biopsy samples to doxorubicin and cyclophosphamide in vitro and survival of the donor patients. Other workers have been less enthusiastic,[12] one of the major drawbacks being the inability of the assay to distinguish between DNA synthesis in tumour and non-tumour cell populations.

The clonogenic assay

All the assays described so far have measured the effect of drugs on the entire cell population of the tumour, which in most cases comprises a mixture of normal and neoplastic cells. The results of these tests could be misleading. It has become apparent recently that even the neoplastic cells in a given tumour are heterogenous in their growth characteristics. Cell kinetic studies indicate that most cells in a tumour turn over slowly, die, or differentiate terminally, only a small percentage of cells being capable of repopulating the tumour—the so called "stem cells." Clearly the best in vitro assay system for predicting response to anticancer drugs should selectively measure the effects of the agent on this small population of stem cells.

Stem cells have been shown to form discreet colonies in agar[13] and two test systems—the Hamburger-Salmon[14] and the Courtenay[15] assays—have been developed to test drug activity against colony forming cells. Both systems take advantage of the observations that fibroblasts which normally "contaminate" tumour cell cultures do not grow in soft agar.

In the standard clonogenic assay cells are incubated for a limited time with varying concentrations of test drug. They are then washed (unless continuous exposure is required) and plated out in agar. After two to three weeks' incubation the number of colonies growing on the plate is counted and the activity of the drug expressed in terms of colony survival in treated plates compared with controls. Colony survival of 30% or less in treated plates is usually associated with in vitro sensitivity and survival in excess of 50% considered to indicate resistance at any single drug concentration.

Human lung cancer cells have been grown in clonogenic systems with specimens taken from primary tumours, bronchoscopic washings, pleural effusions, bone marrow, lymph nodes, and other metastatic sites.[1 2 16–20] Unfortunately, since colony forming cells appear to form such a small percentage of clinical specimens, in many cases clonogenic assays are impossible to perform. In a large prospective study of the clinical usefulness of this form of assay Von Hoff

and colleagues found that only 45 out of 70 lung cancer specimens cultured produced sufficient colonies (>20 per 5×10^5 cells plated) to allow drug testing.[21]

Drug testing using xenografts

Human tumour xenografts become integrated with the vascular system of the host animal and allow in vivo sensitivity testing without disruption of the tumour tissue, in contrast to the in vitro assay. Drugs can therefore be administered in a manner similar to their administration in clinical practice. They are transported by "normal" pathways to the tumour and are subject to metabolic and pharmacological interactions similar to those seen in patients. The drugs can act through several cell cycles and substances that undergo metabolic activation, such as cyclophosphamide, can be tested.

It is unfortunate that as xenografts have gained popularity as a screening system for anticancer agents no standard methodology for assessing drug activity has evolved and become widely adopted. Certain practices have emerged, however.[22 23] Tumour fragments (3–5 mm^3) or cell suspensions (about 5×10^6 cells) are usually inoculated subcutaneously in the flanks of mice. This site offers the advantage that tumour size may be easily measured with calipers. Groups of mice are then randomly allocated to a control group or to a drug treatment, usually at different dosage levels. After drug exposure, caliper measurements are continued and the activity of the drug is assessed and compared in treated and control animals.[23 24]

Growing bronchial carcinomas as xenografts in experimental animals has made it possible to study the chemosensitivity of human lung cancer to various anticancer agents outside the patient.[22 23 25–33] Both single agents and combinations of drugs have been evaluated[30 31] and the model has been used as a basis for the evaluation of novel treatments in human lung cancer.[32 33]

Clinical validity of predictive chemosensitivity testing

The effectiveness of any laboratory assay that attempts to predict the chemosensitivity of tumours in patients ultimately depends on two important criteria. Firstly, the practical aspects of the assay must enable many patients to be tested, with the results available in time to allow prospective planning of treatment. Secondly, the result of the assay must be able to predict the response seen in the patient with reasonable accuracy. Unfortunately, as yet no single assay system adequately satisfies both these points. Many critical articles have reviewed this subject recently.[34–36]

The long latent period after tumour implantation before testing can be attempted makes the use of xenografts totally impractical for determining an individual patient's drug treatment. Clonogenic assays can be performed on only 50–70% of tumour samples and require a satisfactory single cell suspension.[1 21] Theoretically, short term assays of proliferation or viability (for example, radioactive precursor uptake or vital dye exclusion) which do not require single cell suspensions can be performed on a larger percentage of patients (perhaps 90%).[36] Unfortunately these assays may not be as accurate in predicting drug activity as xenografts or clonogenic assays.[12]

The ability of human lung cancer xenografts to reflect the clinical sensitivity of the donor tumour has been extensively studied by Shorthouse.[27 30] These studies are difficult to perform owing to the low success rate in establishing xenografts and the problems of measuring the clinical response. In 16 direct comparisons (seven small cell carcinoma of the lung, nine non-small cell carcinoma) the clinical responsiveness of five small cell tumours to standard combination chemotherapy was associated with highly responsive xenografts. Two small cell patients failed to respond clinically and their xenografts showed similar resistance to the same drugs. All donors with non-small cell carcinoma tumours failed to respond to treatment and the xenografts derived from their tumours were also resistant. These encouraging comparisons have been validated in a smaller study with small cell carcinoma xenografts[37] and with other tumour types.[23]

There are few reports of direct comparisons between the clinical response and in vitro tests using bronchial carcinomas, and no prospective studies have been performed.[34 36 38] Studies so far have shown a prediction of sensitivity in 65–75% of cases (that is, 65–75 out of 100 patients with a sensitive test in vitro had a clinical response) and an accurate prediction of resistance in 90–100% cases. This latter figure appears encouraging but may merely reflect the in vivo resistance to current treatment of most lung cancers. Clearly if only 10% of patients respond to a given drug, then a test which predicted everybody as resistant would be accurate in 90% of cases.[35]

The high level of false positive in vitro predictions is disappointing. It may be partly explained by the results of a recent study[39] in which the in vitro chemosensitivity of cells taken from a metastasis differed significantly from samples taken from the primary tumour in the same patient at the same time.

In reality therefore the prospective use of predictive testing for patients with lung cancer will remain an elusive ideal until rapid, accurate assays are available and can be performed on all patients. Until such time,

these models are an important research tool that should facilitate the development of more effective agents. Interest is currently being shown in Europe in establishing "panels" of xenografts of different histological type and known chemosensitivity to screen promising new compounds for activity. The use of such a system may reduce the work involved in time consuming single agent phase II clinical studies and avoid unnecessary exposure of patients to inactive but potentially toxic agents.

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