HUMAN TUMOUR DRUG SENSITIVITY TESTING IN VITRO

Techniques and Clinical Applications

Edited by
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Foreword

Having had experience of culture studies with human tumours since early days, it is a pleasure for me to be asked to write a preface to this book. I can remember the time when tissue culture resembled somewhat the activities of a club in which white-coated operators carried out mysterious activities behind the closed doors of the sterile room. This was before antibiotics came into general use to hide our mistakes. In those days it was a major achievement to devise a culture medium in which cells could be grown successfully, particularly as cell lines maintained by sub-culture for many years. Those outside the field could not see the relevance of this work either to general biology or to the cancer problem.

However, without this painstaking work by pioneers in tissue culture, the important developments in culture work, which have a direct bearing on the treatment of cancer patients, as described in this book, could not have taken place. It has become evident, both from culture work and from experience in the clinic, that cancers which, according to histological characteristics, are identical, nevertheless are highly individualistic in their sensitivity to drugs; the biochemical or structural factors responsible for these differences are at present largely unknown. In practice, this means that a rapid predictive test on which the clinician could rely would be of immense value for cancer chemotherapy. Culture methods are the only techniques that can provide information within the time that a clinician is prepared to wait before starting treatment.

It is most encouraging to read in this book how a number of clinicians are now working closely with the tissue culture experts. For short-term information and treatment the soft agar method has given strikingly good correlations with clinical results. But the method selects out a small fraction of the tumour population, possibly the fastest growing fraction. It may be necessary, eventually, to develop two types of test, a rapid test and a more detailed examination of the whole spectrum of response of the mixed cell population, possibly using labelling methods and xenografts so that further treatment can be given to avoid reoccurrence of tumour growth.

There is every reason to believe that, as the result of the dedicated work of

biologists, predictive tests will become a standard hospital facility. I think that Dr Hill and Dr Dendy have made a timely presentation of work in this field.

May 1983

E. J. AMBROSE
East Sussex

Contents

1. Introduction

P. P. DENDY and B. T. HILL

It has been recognized for many years that the experimental systems most extensively used to study carcinogenesis, notably continuous cell lines and tumours propagated in animals, have severe limitations as models for human cancers. In particular they fail to take into consideration the marked differences both in the appearance, in terms of histology, vascularity, and stromal involvement, and in the behaviour of tumours in individual patients.

With the advent of cancer chemotherapy it soon became clear that another source of variation was in the response of tumours to anticancer drugs. Furthermore it was apparent that the drug sensitivity of a tumour could not be predicted entirely either from clinical symptoms or from histological examination. Thus a number of groups began to investigate the possibility of using the patient's own tumour material for *in vitro* experiments to predict drug sensitivity (Wright *et al.*, 1957; Di Paolo and Dowd, 1961).

In 1964 Limburg and Krahe reported substantial improvement in the median survival time of patients with advanced ovarian carcinomas if treatment was based on the results of an *in vitro* predictive test. Since median survival time is very susceptible to the presence of one or two very long surviving patients in the group, the improvement achieved with the methods available at that time was probably rather less than the authors suggested. Nevertheless this work gave considerable impetus to the subject and over the next decade a number of short-term culture systems were developed. Disaggregated biopsies containing either single cells or small clumps of cells, tissue explants, or tumour slices were used to establish suspension, monolayer or organ cultures.

Most groups reported evidence of variations in chemosensitivity, and in 1970 Tanneberger and Bacigalupo introduced the term onkobiogramm to describe the characteristic and unique response of tumour cells from one patient to a spectrum of cytotoxic drugs.

In late 1974 a meeting was held in Cambridge (Dendy, 1976) [illegible]

rapidly developing subject of human tumours in short-term culture. The purpose of the meeting was threefold: to report what was already known; to identify technical difficulties limiting progress at that time; to discuss some of the clinical problems that these model systems might help to solve.

It was already clear that a major potential application was the development of predictive assays to characterize the drug sensitivity spectrum of individual tumours. However, a number of outstanding problems were recognized, including:

(i) Can adequate numbers of representative cultures be prepared?
(ii) How should the malignant origin of the cultured cells be confirmed?
(iii) Are the properties of cells in culture a true reflection of their properties *in vivo*?
(iv) Is there sufficient knowledge of the biochemical actions of these drugs for *in vitro* observations to be correctly interpreted?
(v) Is the response spectrum to a given series of drugs always different for cells from specific tumour types?
(vi) Can suitable quantitative assay techniques be developed for the *in vitro* work?
(vii) Is it feasible to make a correct evaluation of the predictive approach in terms of clinically assessed patients' responses, and is the level of collaboration between clinicians and scientists adequate?

Many early publications made little reference to the identity of the cells established in culture, but since 1974 there has been a much greater awareness that such studies must be carried out in parallel with the main experiments. In the first section of this book the various morphological, physical, biochemical, and immunological methods currently available for identification of human tumour cells are presented, and their suitability for recognizing such cells quickly and easily in short-term culture is considered.

A major limitation in 1974 was the lack of a clonogenic assay for freshly cultured human tumour cells. Attempts to extend the pioneering work of Puck and Marcus who cloned HeLa cells as early as 1955 had been unsuccessful. A break-through came in the 1970s with the development of a method that permitted mouse myeloma cells to be cloned *in vitro* (Park *et al.*, 1971) and the subsequent publication by Courtenay (1976) of details of a soft agar assay for solid tumours taken directly from the mouse. The first reported extension of these methods to human tumours was by Hamburger and Salmon (1977) who successfully grew tumour colonies from various human neoplasms including multiple myeloma, non-Hodgkin's lymphoma, ovarian adenocarcinoma, melanoma, and neuroblastoma. Subsequent progress has been extremely rapid and successful colony formation has been reported for many different

human carcinomas and sarcomas. The cloning efficiency is, however, invariably low with sometimes as few as 10 tumour colonies per 500 000 cells plated. For this and other reasons many groups have continued to work with alternative assay methods.

Culture *in vitro* is still unsuccessful with many specimens. Furthermore some researchers consider that growth *in vitro* is too far removed from reality. Therefore other systems have been developed, for example growth in diffusion chambers (Heckmann, 1967; Smith *et al.*, 1976) or as xenografts in immune-deprived mice (Houghton *et al.*, 1977; Bateman *et al.*, 1979). These approaches are most likely to remain research investigations because if a predictive test is to be widely accepted as a routine procedure it must be quick, technically simple, and inexpensive, criteria met realistically only by *in vitro* methods. Nevertheless the major methodological approaches that have been attempted are all considered in the second section of this book, with critical comment on their strengths and weaknesses.

In recent years our understanding of the relevance of certain aspects of cell cycle kinetics to cancer chemotherapy has been greatly extended (see e.g. Hill, 1978) and, as discussed in Chapter 17, we are now much more aware of the need to study the pharmacological behaviour of commonly employed antitumour drugs. Since it is unreasonable to suppose that the *in vitro* drug concentration, expressed as a function of time, will imitate *in vivo* behaviour, deductions must be based on differences in relative sensitivity between different tumour specimens treated with the same drug under standard conditions. Nevertheless it is reasonable to select a range of doses for testing *in vitro* on the basis of the dosage that can be achieved pharmacologically.

Clinical investigators will have a particular interest in the final section of this book which concentrates on correlations of laboratory predictions with clinical response to treatment. It is encouraging to report clear evidence of increasing cooperation between clinicians and scientists, and an increasing tendency, within the requirements imposed by the needs of individual patients, to adopt a more uniform approach to testing and evaluating chemotherapy regimens. This greater degree of standardization is essential if valid deductions are to be made from the data. However, the breadth of contributions in this section reflects once again the current broad spectrum of opinion over the best approach to this problem of predictive drug sensitivity testing.

Throughout this book contributors have adopted a critical approach and, as a result, the reader may find conflicting views expressed. We make no apology for this. Indeed we hope it will help to emphasize that the present position is fluid and many problems remain to be resolved.

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Part I
Tumour Cell Identification in Biopsy Specimens, in Mixed Culture and in Isolated Colonies

2. Morphological Criteria for Tumour Cell Identification

L. M. FRANKS

I. Introduction

There are no features that absolutely identify every tumour cell, and a few moments thought about the nature of the neoplastic process makes it plain that this objective is not likely to be achieved. Alterations in structure and in behaviour appear at a relatively late stage in this process. In an experimental situation, with a known carcinogen, the initiating event which follows the application of a carcinogen usually produces no change that can be identified as neoplastic, nor do such changes appear during the greater part of the long subsequent latent period, yet the treated cells have been altered and can be induced to form tumours by the application of promoting agents. Even after morphologically altered cells appear, there may be no direct relationship between the degree of structural alteration and the "malignancy" of the cells. Although well differentiated tumours usually are less "malignant" than more anaplastic tumours, this is not invariably the case. Some well differentiated "grade 1" tumours may metastasize while other apparently more "malignant" tumours may not. Over the years a pattern of tumour structure and behaviour has been established. To take a simple example, confident predictions can be made if a lump in the breast of a woman is found to have a specific microscopic structure, e.g. grade 1 adenocarcinoma. Experience has shown that 80% of women with this particular pattern will be alive 5 years later although 20% will be dead. The pattern and structure will not allow a distinction to be made between individuals in the two subgroups, i.e. morphology alone is not enough. Even this behaviour pattern is only identifiable in a group in which there is clinical evidence of tumour growth, i.e. a lump. In circumstances in which the morphological change is found by chance no prediction can be made. A classic example of this situation is found in the latent carcinomas that occur so frequently in the prostate (Franks, 1956). These lesions, although they have the morphological structure of tumours, are considered by some not to be true tumours since they do not appear to be growing. Hence there is the

need for methods to measure growth (or growth potential). Logically tumours that are growing most actively in the host would be expected to grow most rapidly in tissue culture. Unfortunately this too is not the case, and again there seems to be no direct relationship between structure *in vivo* and growth rate *in vitro* or even the ability of tumour cells to grow at all *in vitro*. On the relatively small proportion of cells that will grow *in vitro* a number of behavioural tests can be applied. Results of some of these can be assessed by alterations in structure, and some may be correlated with "malignancy". Morphology alone can identify altered cells. Can functional morphology identify cells with increased or decreased growth potential or other characters associated with "malignancy"? There are many reviews on markers of neoplastic transformation (e.g. Cameron and Pool, 1981; Busch and Yeoman, 1982; Franks, 1979, 1982) but most of these markers have been established using mesenchymal cells transformed *in vitro*, whereas the common tumours are epithelial in origin. In most experiments changes in cells grown from normal tissues have been recorded after the application of a transforming agent. The well known appearance of "transformed foci" of irregular fusiform cells in a random criss-cross pattern was first described in experiments of this sort and is regarded as a reliable indicator of transformation. Unfortunately a careful study (Sanford *et al.*, 1974) showed that, although some treated cultures contained fusiform cells growing in a random criss-cross pattern, these cultures did not form tumours in untreated hosts. Most of the cell lines that developed from carcinogen-treated cultures and were tumour-producing showed neither a fusiform shape nor a criss-cross pattern. Others (e.g. Tomei and Bertram, 1978) have shown that this kind of transformed phenotype can be returned to normal (as can contact inhibition of growth) by growing the "transformed" cells in chemically defined medium, so that appearances in culture seem to reflect environmental conditions. Reversion to the transformed pattern could be obtained by adding 2% serum or 0.1% albumin to the medium in the log phase of growth or by exposure to trypsin for 30–60 s. Other markers for transformation, e.g. growth in agar, fibronectin production, cytoskeletal changes, etc., also show a dissociation between the presence or absence of the markers and malignancy, especially in epithelial systems (see, for example, Marshall *et al.*, 1977; Franks, 1982, for review). Some of these problems have also been discussed in detail (Franks, 1979). Here they will be considered in the specific context of identification of tumour cells. Most of the commonly used markers require mass populations of cells, so methods that can be applied to single cells would be invaluable.

II. Tumour cells *in vivo*

The morphology of tumour cells has been described and illustrated many