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Editorial

Human Tumor Clonogenic Assays

An Overview

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In this issue of the Journal, we present a collection of papers on human tumor clonogenic cell assays and their potential use in cancer chemotherapy, both for predicting clinical efficacy of standard and new agents and as a tool for studying the cellular pharmacology of spontaneous human cancers. These papers were presented at a conference held by the Basic Science Program of the Northern California Cancer Program in Palo Alto, California, on 16 October 1980. The papers are presented in essentially unedited form and are meant to represent the state of the art in a rapidly evolving field rather than a finished body of work. They are especially useful in that they demonstrate the diversity of approach by groups working in this area as well as some common threads.

Clonal growth of mammalian cells was first achieved 25 years ago [20]. Many experimental animal tumors and cell lines have since been shown to form colonies under various plating conditions, with efficiencies ranging from 20%–80% [5, 8, 14]. These clonogenic techniques have been extremely useful in studies of the effects of various cytotoxic agents. Experiments with spontaneous human cancers were discouraging, however, since many such tumors failed to form colonies in vitro, and only rarely was a plating efficiency above 1% observed.

Interest in the use of clonogenic assays to study human cancer cell growth and drug sensitivity has been revitalized by the work of the Tucson group [1, 15, 22]. Their principal contribution initially was to demonstrate that it may be possible to extract important information by using plating efficiencies in the order of 0.1%–0.01%. As a result of these observations, the technique is now being widely studied, both to further develop methodology and to attempt to validate the original premises. At the same time, progress continues to be made in other cell systems where the goal has been (a) to optimize growth conditions, thus increasing plating efficiency; and (b) to maintain in vivo characteristics of the cells. The papers from Rosenblum's and Smith's groups are particularly interesting in this regard [21, 24].

Much of the motivation for the development of these assays arises from the frustrations of the clinical oncologist. In the large majority of human cancers, so-called active drugs, used as single agents rather than in combination chemotherapy, produce measurable but brief tumor shrinkage in 20%–30% of cases. Almost all patients, however, will experience toxicity from these drugs. The results of second-line treatment after failure of an initial chemotherapy regimen are even more dismal. This is illustrated by data on ovarian carcinoma from the MD Anderson Hospital [24]. Patients treated initially with melphalan had a 25% objective response rate in this series, but after failure of treatment with another agent, melphalan produced only an 11% response rate. The same pattern of development of broad cross resistance was observed with other drugs used alone and in combination. It is obvious that any method which could reliably predict drug sensitivity or resistance in individual patients would have potentially important clinical applications.

Work with human tumor clonogenic assays can be summarized by these general postulates:

1) Cells from most spontaneous human tumors can form colonies in soft agar, albeit at low plating efficiencies;

2) These clonogenic cells are representative of tumor stem cells which are responsible for sustained malignant growth in vivo;

3) Statistical correlations can be devised to relate drug-induced cytotoxicity in vitro and clinical response to chemotherapy.

With regard to the first two postulates, it is now well established that 50%–70% of human cancers

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will form colonies in semisolid media, with plating efficiencies usually of 0.1%–0.01% [2, 16, 23, 27]. This is true for a wide variety of carcinomas and sarcomas. Lymphomas are an exception, with plating efficiencies of at least 0.01% achieved in only a small percentage of cases.

A major concern is that the cells which grow at such a low plating efficiency may not be truly representative of all stem cells in the tumor. In this regard, the true percentage of stem cells in human cancers is almost certainly much higher than 0.1%. Plating efficiencies of 1%–15% have been achieved for many human tumors in enriched systems, such as diffusion chambers in mice and feeder layers [9, 10, 25].

The percentage of potentially clonogenic cells may be even higher in vivo, where cancer cells grow as tissues rather than isolated cells, and are subject to cellular interactions, stromal elements, and influences of growth factors, all of which are still poorly understood.

The ultimate test of whether these clonogenic cells are representative with regard to drug sensitivity is the strength of the correlation between in vitro effects and in vivo treatment results.

The available data would indicate that, for a limited number of different tumors and drugs, the predictive power of the assay is 60%–70% true-positive for sensitivity and 94%–98% true negative for resistance [1, 2, 22, 27]. However, the data for resistance are derived largely from retrospective correlation of the in vitro results in patients who have been exposed to a drug and failed treatment. Testing resistance after drug exposure may not yield the same correlations as prospective studies, and we feel these should be considered separately.

Ideally, such correlations should be prospective, with in vitro sensitivity testing performed prior to treatment with a single drug. Clinical protocols utilizing the assay prospectively are subject to ethical constraints and review, which may make it increasingly difficult to obtain prospective correlations of resistance. It should be pointed out that the criteria for sensitivity in vitro are arbitrary and reflect the clinical desirability of keeping false-negative correlations to a minimum (i.e., resistance in vitro to a drug that may be effective in vivo). These criteria are at present based on a relatively small number of tumors and drugs. Both the area under the survival curve at a few drug concentrations and the percentage of surviving colonies at a given drug concentration have been used [17, 27].

Major changes in assay methodology may be expected to change these sensitivity criteria and correlations, and it is incumbent on each group to validate such changes. A large data base will be required, and criteria for prediction of drug sensitivity may be different for each drug and perhaps for each tumor type [17].

In this regard, the papers presented in this symposium represent numerous variations in technique, some major and some minor. For example, a group concerned especially with optimizing the growth of normal and malignant breast epithelium achieves high clonal plating efficiency *after* propagating cells initially from clusters, using a conditioned medium and fibroblast feeder layers [24]. In this system, normal epithelial cells grow as well as breast cancer cells, a disadvantage if one is concerned primarily with the drug sensitivity of the malignant cells in the mixed population of a surgical specimen. The high plating efficiency, however, may offer marked advantages in the study of small tumor samples and also resistant subpopulations within a tumor. Comparison with the 'standard' soft agar system and prospective clinical correlations will be essential when major modifications in methodology are introduced.

The ability to clone normal cells emphasizes the necessity to characterize colony-forming cells routinely in each assay by various techniques, including histologic, immunochemical, and karyotypic methods. Normal inflammatory cells, myeloid precursors, and cells from benign tumors have been shown to form colonies under the conditions used for human tumor clonogenic assays [4, 22].

Most groups are testing either a range of drug concentrations, or a single concentration which is 10% of the achievable in vivo drug concentration at standard clinical doses [3, 27]. Exposure of cells to a range of concentrations may detect patterns of drug sensitivity that are not evident with a single drug concentration [18].

Usually, colonies are defined as aggregates of greater than 30 cells [22, 27]. Some groups, however, are using simple size criteria, namely either 50- or 75-μm diameter cell aggregates. Inclusion of clusters of 15–30 cells may substantially alter observations of cytotoxic effects [12].

It is clear that these various techniques are evolving rapidly, and attempts are being made to optimize conditions for individual tumor types [6, 7, 21, 24]. Combined mechanical and enzymatic disaggregation improves cell yield and viability over those obtained with mechanical disaggregation alone, but the effects of various enzyme pretreatments on drug sensitivities have not been systematically examined [19, 23]. Many other factors related to in vitro growth conditions, individual drug characteristics, and in

vivo pharmacological factors will affect the eventual clinical validation and correlations [3, 13, 21].

In addition, the techniques may prove to be of major benefit ultimately to only a small fraction of cancer patients. An adequate tumor specimen is frequently not available from patients, not all tumors will grow sufficiently for drug sensitivity testing, and among the tumors which do grow, 'clinically significant' sensitivity may be detected infrequently.

Despite these reservations, human tumor clonogenic assays are an exciting new development in oncology. In addition to potential clinical applications for both established anticancer drugs and agents undergoing development, the clonogenic assay provides another tool for studying the cellular pharmacology of human cancers, including studies of tumor cell heterogeneity, mechanisms of drug resistance, and drug interactions. The diversity of methodologic approaches and applications is illustrated in the papers which follow.

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