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Perspectives and Commentaries

Recent Improvements in the Human Tumor Cloning Assay for Sensitivity Testing of Antineoplastic Agents

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IMPORTANT goals in the treatment of patients with malignant diseases are to minimize the side effects of chemotherapy and to optimize the chance for prolonged survival. Several attempts have been made over the past decades to develop *in vitro* assays to help predict sensitivity or resistance of an individual patient's tumor. These *in vitro* assays might also help define the activity of new, experimental chemotherapeutic agents.

Probably the best characterized *in vitro* assay is the human tumor cloning assay (HTCA), first described by Hamburger and Salmon [1, 2, for review: 3]. To perform this test, a biopsy (or effusion) containing tumor cells is taken and a single-cell suspension prepared. The cells are exposed to various antitumor drugs and then seeded in soft agar. A number of tumor cells will proliferate in this environment over the following 1–2 weeks and will eventually form colonies. The number of colonies formed by drug-treated cells is then compared with colony formation by control cells to assess the antiproliferative effect of the agents.

Over the past years, the clinical correlations of assay results have been investigated in retrospective and prospective studies. The results indicate a 60–80% chance for complete or partial response when patients are treated with a drug that inhibits colony formation in the HTCA. If, however, a drug is inactive in the HTCA, the chance for a clinical response is only 5–15% (i.e. with a 85–95% probability the tumor will be clinically resistant to treatment with this drug). The impact of HTCA-guided chemotherapy on patient survival is currently not clear. However, some reports suggest a survival benefit in patients with ovarian cancer. Other tumor types have not been studied as extensively yet [4, 5].

Can the HTCA then be used in the routine clinical management of patients and planning of chemotherapeutic regimes? So far, the answer has been no because of technical problems [6]. However, advances that improve assay reliability have been made. These advances include:

1. *Improvements in quality control.*

Of crucial importance for a correct interpretation of assay results is the presence of a good single-cell suspension at the time of plating [7]. Thus, besides a "negative control" for cell kill, consisting of the drug diluent, each assay should now include a "positive control" with a highly toxic compound. The purpose of the "positive control" is to make possible the detection of cell clumps that might be erroneously counted as tumor colonies. Various procedures for positive controls have been examined including irradiation or refrigeration of plates and the addition of highly cytotoxic agents like glutaraldehyde and sodium azide. In our laboratory, the antitumor antibiotic chromomycin A3 or ortho sodium vanadate at high concentrations have been successful positive control agents [7, 8].

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2. *Decrease in number of cells required for drug testing.*

An important limitation not only for the HTCA but all *in vitro* systems is the number of cells available from the patients' tumor. Quite often, lack of cells prevents testing of a large enough number of drugs to find an active agent. Considerable progress has been made by the introduction of a capillary technique into drug testing in the HTCA [9, 10]. This system was originally developed for granulocyte-macrophage colony growth by Abrams *et al.* [11] and has been used for the study of bone marrow colonies by Maurer [12]. Its application for primary human solid tumors has many advantages. Due to the smaller volume of agar, the number of cells needed to perform the HTCA is only 1/5 of the cell number needed for the conventional HTCA in 35 mm Petri dishes. In addition, the amount of drug used for each test is reduced to about 1/3. This is important for the screening of new agents which are only available in small amounts. Other advantages are a lower risk of contamination as compared to plates and a 3 to 30 fold increase in cloning efficiency [9, 10, 12]. One still unresolved problem in the capillary system, however, is the time-consuming counting procedure. Counting equipment to facilitate the evaluation of colony formation is currently being evaluated.

As we have pointed out, the final value of any *in vitro* method depends on how accurate the assay predicts for the clinical outcome (for review see 13). It is important to keep in mind, that changes in the assay technique might alter the predictive power of the assay and, from a theoretical point of view, the usefulness of a modified assay for the clinical setting needs again to be demonstrated. A prospective and randomized single agent trial at our institution demonstrates an increase in the response rate of patients treated with HTCA-guided chemotherapy as compared to the physician's choice of a single agent. These results are encouraging since patients in our study had far advanced disease and often had received chemotherapy with multiple agents prior to entry into the study.

3. *Improvements in growth conditions.*

A major technical problem with the HTCA is poor growth of tumor specimens in soft agar. Available figures vary widely depending on the tumor type and the definition of growth. Recent investigations with a variety of growth factors indicate that the *in vitro* growth of tumor cells can be improved. The growth factors successfully tested include Epidermal Growth Factor and Alpha-Transforming Growth Factor [14, 15]. Polypeptide hormones like insulin as well as steroid hormones (hydrocortisone, estradiol) have also been reported to increase colony growth [16]. However, negative reports on the effectiveness of hormone or growth factor supplements have also been published [17]. At present, the results are too preliminary for final conclusions. Although some progress has been made to define the growth requirements of malignant cells *in vitro*, the use of defined media has not been successful. Clearly, more work in this respect needs to be done before growth conditions can be optimized and controlled.

4. *Improvements in time requirements.*

The use of precursor-uptake and -incorporation techniques in the HTCA has been reported to decrease the incubation time significantly and still give accurate results [18, 19, 20]. Drug sensitivity data can be available after 5–7 days as compared to 2 weeks with the conventional assay. A drug-mediated decrease in ^{3}H-thymidine incorporation of 80% is believed to be equivalent to a 75% decrease in colony formation [18]. Also, precursor incorporation techniques have been reported to substantially decrease the number of cells required for drug testing [20].

These new methods would also benefit from a prospective clinical trial.

Besides its potential usefulness for planning of chemotherapeutic treatments of cancer patients, the HTCA has shown promise in the detection and development of new anticancer agents. Currently, the National Cancer Institute screens about 10000 compounds each year in murine systems for antitumor activity. Although this is an efficient way to cope with the myriad of chemical compounds there might also be disadvantages. Drugs that are inactive in murine leukemias might still be active against human primary solid neoplasms. In fact, a recent combined study of four major centers, under the sponsorship of the National Cancer Institute, demonstrated that, of 79 compounds which were negative in the NCI-screen (P388 mouse leukemia), 14 (18%) had antitumor activity in the HTCA. This is indicating that the HTCA might detect new types of compounds and might prove to be a valuable *in vitro* test in the development of anticancer drugs if further advances in assay techniques can be achieved.

In summary, the HTCA has still to be considered an experimental method. Well planned clinical randomized studies now in progress will help define the exact value of the HTCA in patient management. Measures to increase the number of tumors with growth in the HTCA will increase its usefulness in drug development.

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