Drug response assay: an increasing trend in designation of tailored-chemotherapy for more rational management of cancer patients

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Drug response assay, in other words tumor chemosensitivity assay, has long been known but technical difficulties regarding the performance of the assay in the past caused a significant drawbacks. However, recent technological achievements (e.g. luminometric measurements) seem to overcome these difficulties. Therefore, individualization of drug treatment via drug response assay may again take its place in oncology clinics. Promising results regarding the ATP-tumor chemosensitivity assay are reported especially in the treatment of recurrent ovarian carcinoma. Although its widespread use in clinics needs more randomized studies, the trend in using the assay could be expected to develop fast in parallel with the increasing number of chemotherapeutic agents that are coming in to market.

Key words: Chemosensitivity, ATP-TCA, drug response


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Introduction

Although many chemotherapeutic drugs have been introduced for last 40 years, it seems that there is still no satisfactory improvement in the successful treatment of cancer patients. This is mainly because cancer is a heterogenous disease, which means responses to same anti-cancer drugs can be various from patient to patient even though they all develop the same organ-originated cancer with histologically same phenotype. Because of this fact, it is not likely to expect the same outcome in all the patients who are treated with the same drug(s).

The systemic treatment of malignancies has been based on physicians' empirical judgement, relying on data obtained from clinical trials. However, even histopathologically identical tumors behave so differently that the response rate of tumors to the chemotherapeutics was, and still is, sometimes greatly varied. These variations may result in failure of the treatment of cancer patients, or even toxicity which leads to the death of the patients. As it is known, chemoresistance is a key feature of success in cancer treatment. In fact, this is one of the nightmare scenarios which may be faced up during treatment of cancer. Therefore, to know the resistance or sensitivity in advance is of immense importance in the success.

The effectiveness of current therapeutic approaches is limited mainly by tumor heterogeneity, as it is known. That is why one of the aims of basic cancer researchers is to predict the response of patients with cancer to chemotherapeutic agents by employing laboratory methods variously called 'tumor chemosensitivity assays', 'drug response assays', or 'drug sensitivity assays'.

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The tumor chemosensitivity assay (TCA) can be used as a tool to let oncologists know about which anticancer drugs are more likely to work well enough (or not) on their patients. It is believed that the response rate, the progression free survival as well as the overall survival rate of cancer patients could be improved by performing such a test. More benefits are also expected, as stated below. There are a number of tests being used for this aim as briefly explained in the next sections. To perform the test, the cancer tissue removed from patients during routine surgical operation should send to the laboratory in a medium. After that, as many as up to 20, even 30, different drugs are incubated with the cancer cells isolated from the cancer tissue in order to determine their cell-killing potency.

Prediction of response or overall survival is of immense importance in oncology practice. Therefore, there is no doubt that oncologists would like to make these predictions as early as possible. It would not be an overreaction to say that every oncologist has eagerly this wish. Detecting the chemosensitivity and/or chemoresistance of tumor tissue freshly removed from the patient during routine surgical operation to anticancer agents in vitro has appeared to be an attractive way of getting this wish come true. TCAs have already been in use in the field of oncological research and these tests should be able to fill the gap in predictive oncology. There is an increasing trend to develop new methods with this aim.1 A working tumor chemosensitivity assay would be of immense benefit to the pharmaceutical industry, and to oncologists and their patients.2 These assays could also be used to design new treatment modalities, as has been reported for recurrent ovarian cancer in a very recent paper.3 The first experiments regarding this issue date back to the early 1950s4,5, but little attention was paid until the well-known paper published in the New England Journal of Medicine in 1978.6 The attention was taken a bit further in the 1983 report published in the same journal by Von Hoff.7

Tumor Chemosensitivity Assays

Since the 1950s, a number of methods for assaying tumor chemosensitivity and/or chemoresistance have been developed. Some well known TCAs are listed in Table 1.

The major applications of TCAs include a. assessment of sensitivity and resistance, b. optimization of therapy for efficacy, toxicity and costs, c. designation of therapy for pretreated/relapsed tumors, d. chemotherapy of metastases of unknown primary tumors, e. chemotherapy of rare tumors. In addition to these, there are more benefits, such as a. evaluation of mechanisms for resistance modulation, b. evaluation of drug interactions on cellular level, c. evaluation of new substances, combinations, and principles, d. evaluation of therapeutic index.

In chemosensitivity assays, there are mainly two approaches: using ready-to-use cell lines or preparing up primary cultures. The cell lines have traditionally been used by pharmaceutical companies. Cell lines have some advantages over primary cultures (e.g. shorter assay time, technically less complication, less labor, quicker to perform)21 but they are often found to be poorly representative of the behaviour of “real” tumor cells.22 Therefore, using primary culture, instead of commercially available cell lines, are of immense importance in drug evaluation assays. The common feature of these methods (either for cell lines or primary cultures) is basically the employment of cell culture techniques. Both types differ in their processing.

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Table 1
Major tumor chemosensitivity assays
and the technique used to measure sensitivity/resistance. All those techniques involve 4 basic steps: 1) isolation of cells; 2) incubation of cells with anticancer agents for a certain period of time (2-6 days); 3) assessment of cell viability; and 4) interpretation of the result.

Among the methods listed in Table 1 above, the human tumor stem cell assay (human tumor clonogenic assay) is one of the first methods used as a TCA. Hamburger and Salmon used it to test the effects of drugs on tumor tissues in 1970s. Subsequently, Von Hoff improved it to the degree of a proper TCA. However, clonogenic assay (or its derivatives) measure the ability of drugs to inhibit tumor cell proliferation capacity, which is believed to result in the abolishment of these methods currently. The extreme drug resistance assay, developed by Kern and Weisenthal, is a method in which tritiated thymidine is used to measure the DNA synthesis rate. It is mainly used for the measurement of resistance of proliferating cells to the drugs given. Therefore, it is also called "extreme drug resistance assay" or "cell culture drug resistance testing, CCDRT". In this assay, it is assumed that thymidine incorporation into DNA reflects cell division and does not occur in non-tumor cells that contaminate the biopsies. This could, to some extent, seem to be advantageous of this assay but due to its inability in dealing with non-proliferating cells that could present in tumor tissue, this assay was also considered not to provide a reliable quantification of drug sensitivity in tumor samples. The differential staining cytotoxicity assay (DISC Assay) is a method for measuring apoptosis. One of the major modes of action of chemotherapeutic drugs is known to be via the activation of apoptosis, which is a fundamental mechanism of cell death that can be engaged by a range of cellular insults. It is thought that understanding how the cell death program is initiated by following these drugs or any other kind of insults, and hence why it fails to work for certain drugs, offers a novel approach to overcoming the clinical problem of drug resistance. DISC Assay is performed by staining of cells followed by the microscopic evaluation. In this assay, a dye, such as fastgreen, is used, which enters only non-living cells. The ratio of dead cells to total cells allows to evaluate cell death. Kaspers stated that although it was laborious and requires skilled technician, it requires few cells, the assay can be used for proliferating and non-proliferating cell populations, and can discriminate between malignant and contaminating non-malignant cells. The tissue Explant Assay (Histodrug response assay, HDRA) relies on the three-dimensional architecture of the tumor. It is also known as histoculture drug response assay (HDRA) in which a piece of tumor is grown in collagen bed. Thus, this method demands as much tissue as possible. The cell survival is measured by MTT reduction that is the principle of the MTT assay. It was reported that chemosensitivity determined by the HDRA seems to be a strong predictor of survival in patients with advanced HNSCC and should be considered further. The fluorescent cytoprint assay (FCA) uses a fluorescent dye that is converted in living cells. This assay does not require cell dissociation. Tumor fragments are maintained in a collagen matrix. A fluorescent dye accumulates in living cells and then visualized and photographed as "cytoprints". The MTT assay was originally developed by Mosmann and is one of the most commonly used methods to measure the tumor chemosensitivity in order to predict the drug response. The MTT assay determines the ability of viable cells to convert a soluble tetrazolium salt, the MTT compound, to an insoluble formazan precipitate. The formation of formazan is positively correlated with the number of viable cells in the culture. The MTT assay is a rapid colorimetric and quantitative method and it is usually regarded as reproducible and suitable for initial-stage in vitro drug screening. Nevertheless, the MTT assay may suffer some interferences from some of the chemicals (e.g. N-acetyl-L-cysteine) used frequently for various reasons in cell culture environments. In a recent study, the MTT assay was therefore checked in terms of its possible chemical interactions with 22 different anti-cancer drugs presently used for cancer treatment, including the frequently-used ones, such as cisplatin or etoposide. Ulukaya et al in the same study concluded that before employing the MTT assay, drugs (or any kind of substances) to be included in the assay should be checked first in terms of possible chemical interactions with MTT otherwise it might be impossible to evaluate the MTT viability assay results correctly.

ATP-Tumor Chemosensitivity Assay (ATP-TCA)

It seems that there is an increasing trend in the utilization of the tumor chemosensitivity assays. In a recent report, a 3-dimensional ex vivo culture technique was stated to be a useful method for drug development and the prediction of in vivo drug efficiency.
ATP-TCA is one of the most recent methods for testing tumor response to drugs in addition to chip-based methodologies in which some other parameters of cell survival (e.g. pH) are monitored and measured at the same time. There is an internationally recognized society for the chemosensitivity testing studies and it is called ISCO (The International Society for Chemosensitivity Testing in Oncology). The ATP-TCA is created by particularly Prof. Dr. Ian Cree (Portsmouth, England) and his co-workers who are founders of the ISCO. In ATP-TCA, tumor cells are cultivated in vitro for 6-7 days with titrated concentrations of chemotherapeutic drugs to evaluate tumor sensitivity and resistance. Microwell culture plates and serum-free culture medium which support the selective growth of tumor cells are used to perform the assay. Its serum free property is a unique feature. This is an important issue because medium used for cell culture often provide considerable growth stimulation, either by the addition of serum or artificial supplements. Therefore, it was reported that testing of tumor-derived cells in serum free medium providing less growth support could have a number of advantages in targeting drugs to the most appropriate tumors.

Drug inhibition of tumor cell growth is quantified using a luminometer to measure the light emitted when adenosine triphosphate (ATP) from the cultured tumor cells is converted in the D-luciferin-luciferase bioluminescent reaction:

\[
\text{ATP-TCA} \rightarrow \text{AMP + 2Pi + Photon}
\]

The ATP-TCA seems to show a significant promise in evaluating the anti-cancer potential of chemotherapeutic drugs against cancer cells isolated from many different kinds of solid tumors. More importantly, it has also reached a phase III clinical trial (in recurrent ovarian cancer), of which results might make a great impact on cancer treatment in near future. ATP-TCA has also been evaluated on many other tumor types.

ATP-TCA utilises serum-free medium called CAM (complete assay medium) which promotes the viability of tumor cells while it inhibits the growth of normal/stromal cells. Selective tumor cell growth is also encouraged by the use of polypropylene microplates which do not permit cell adherence. Its ability to select the survival of cancer cells was shown in 124 specimens under the serum-free conditions by Andreotti et al. They tested 39 breast tumors, 78 ovarian tumor or ascites specimens, and 7 other solid tumors. They reported that the mean proportion of malignant cells was 54% (10-95% ± 22%) before culture, while it increased to 83% (30-100% ± 14%) after culture, resulting in the mean increase in the percentage of malignant cells before and after culture being 29%.

ATP-TCA results should be regarded as evaluable if three criteria are met: 1. clear evidence of malignant cell involvement of the sample by cytology or histology, 2. MO (no drug control) values greater than 20 000 RLU indicating the presence of enough number of alive cells in culture system, and 3. evidence of a dose relationship. In the analysis of results, percent tumor growth inhibition (TGI) is calculated for each test drug concentrations (TDC). The calculation is performed as formulated below:

\[
\text{TGI} = 1.0 - \left( \frac{\text{TDC} - \text{MI}}{\text{MO} - \text{MI}} \right) \times 100,
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where MO = mean counts for no inhibition control cultures, MI=mean counts for maximum inhibition control cultures, and TDC=mean counts for replicate test drug cultures.

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ATP-TCA seems particularly to be useful in ovarian cancer. In the study of Konecny et al [35], they reported a significant correlation between drug sensitivity/resistance and clinical response (p=0.007). In the same study, the assay demonstrated a sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of 95, 44, 66, and 89%, respectively. The NPV in this study is especially notable because it gives an appreciable idea of the patients who will show recurrence within 12 months of post-chemotherapy period. Out of 9 patients, 8 were found in vitro resistant on the basis of sensitivity index, according to the assay. In other study, regarding the cisplatin resistance, it was found that the assay had 90% accuracy for cisplatin resistance of ovarian carcinoma. ATP-TCA has also been evaluated in a pilot study of recurrent ovarian cancer in which therapy was selected on the basis of ATP-TCA results by Kurbacher et al. In the study, 25 patients were treated according to the ATP-TCA results while 30 patients were treated empirically. It was shown that therapy guided by ATP-TCA produced a greater benefit with regard to both overall response rate (ORR) and progression-free survival.
(PFS) in platinum-refractory patients. Kurbacher at all also stated that ATP-TCA could be regarded as the most sophisticated assay to investigate both solid tumor samples and effusions derived from patients with various organ tumors [36]. In another study, prospective studies are needed to be routinely used of ATP-TCA in selecting drug therapy, although it was regarded having a predictive ability for drug response.37

In addition to this benefit in selecting the best regimen for particular patients, ATP-TCA was also reported that it could succesfully be used for new agent development [20]. Thereby, in the same report, Cree at al suggested that ATP-TCA should limit the number of subsequent phase II/III trials required, saving money and time, while permitting new agents affective for subsets of cancer patients to be introduced faster. There are some conclusions of early clinical trials (oral communication with C. Sartori, Germany) regarding the ATP-TCA in recurrent ovarian cancer that are given below: 1. The ATP-TCA provides promising retrospective ex vivo/in vivo correlations; 2. Both the positive predictive value and the predictive accuracy of the ATP-TCA compares favorably with other predictive assays; 3. A multi-parametric evaluation score including different informations drawn from the dose-response curves appears to be superior to uni-parametric approaches (IC50, IC90, sensitivity index etc.); 4. The ATP-TCA provides a rational approach for preclinical evaluation of novel active combination regimens for ovarian cancer (preclinical phase II studies); 5. Preclinical phase II trials are likely to identify indicators for subsequent clinical phase II studies and thus appear to be a time- and cost-saving approach in the development of new chemotherapy protocols; 6. ATP-TCA-directed chemotherapy for recurrent ovarian cancer produces both impressive response rates and promising long-term results (PFS and OAS), as compared to empirical salvage chemotherapy; 7. Responses seen with ATP-TCA-based chemotherapy are often of high quality (CR); very few patients (false positive results) progressed on ATP-TCA-directed chemotherapy; 8. ATP-TCA-directed chemotherapy appears to be of particular value for platinum-refractory patients; the chance to experience long-lasting PFS and OAS is increased to a level normally seen only for platinum-sensitive individuals; 9. Standard platinum-based protocols seem to be not always the best choice for patients with platinum-sensitive disease, whereas patients with refractory tumors may often benefit the most from protocols others than single agent paclitaxel; 10. The ATP-TCA is able to identify patients to benefit from novel regimens which are normally not chosen empirically but accounted for more than 50% of responses seen; 11. Randomized studies are warranted to confirm the promising results of the trials.

Conclusion

In brief, it seems that there are two great benefits to expect from TCAs: Firstly, exclusion of chemotherapeutic agents which are not likely to be effective, thereby avoidance of their potential toxicity. Secondly, selection of chemotherapeutic agents with the greatest likelihood of clinical effectiveness for improved response rates and prolonged survival. In addition to these two great benefit, there are more advantages of using TCA which are briefly mentioned below. 1. Relative efficacy of two similar drug or drug combinations is elucidated; 2. When standard therapy fails, newer or non-standard therapies can be considered; 3. A therapeutic index can be obtained when normal cells are also tested; 4. Financial savings are possible as expensive new drugs can be avoided in test-resistant patients; 5. Sensitivity to radiotherapy can also be measured. TCAs should therefore deserve some attention in order to better management of cancer patients and need to be evaluated in prospective randomised studies. TCA, at least, currently seems to gain a quite respectful position in the treatment decision of recurrent ovarian carcinoma (oral communication with S. Lele, USA).

References


