Rational therapeutics

Customizing therapy for a disease based upon the best drug or group of drugs for an individual patient is a therapeutic approach on the increase. The approach has been routinely used for many years for difficult-to-treat bacterial infections. The offending microorganism is isolated and tested against a battery of antibiotics to select the optimal treatment regimen and to avoid those drugs to which it is resistant. More recently, the same strategy, but with a genetic twist, has come into use for the treatment of HIV infections. Now, the latest development in customized therapy is for cancer patients. Their tumor cells are tested for sensitivity and resistance to anticancer drugs *in vitro* as a guide to the appropriate drug combination for use in chemotherapy. These new strategies for designing effective drug therapies on a patient-by-patient basis, in contrast to the use of a standard, off-the-shelf therapeutic regimen, are likely to have a major impact on the use of existing drugs and the development of new therapeutics.

Customized chemotherapy

In the case of HIV infection, individualized chemotherapy became realistic once the new DNA chip technologies made it possible to sequence a viral genome quickly and routinely in order to identify the particular HIV strain infecting the patient[1,2]. The GeneChip HIV PRT Assay, marketed by Affymetrix Inc. (Santa Clara, CA, USA) and developed in collaboration with Glaxo Wellcome plc (Stevenage, UK), is designed to quickly sequence the HIV *pol* and *pr* genes. By knowing the relationship between specific mutant versions of the HIV reverse transcriptase and protease (enzymes encoded by the *pol* and *pr* genes) and their susceptibility and resistance to specific drugs, physicians can now select the most effective therapeutic regimen and avoid those drugs to which the virus is resistant. This approach to HIV therapy has been a major advance, allowing the physician to quickly find an effective treatment strategy without the trial and error drug selection process that often leads to increased drug resistance.

Customized chemotherapy for cancer patients is not a new idea. In fact, it is an old idea that was tried and discarded in the early 1980s. Despite these past failures, Rational Therapeutics, a small company in Long Beach, CA, USA, was formed on the basis that it is possible to identify the very best chemotherapy regimen for a given cancer patient, and avoid using drugs to which the patient's tumor cells are resistant, by evaluating *in vitro* the effects of various drugs on that individual's tumor cells.

Dr Robert Nagourney (Medical Director of Rational Therapeutics) says the reason that his company exists when others have failed is because the earlier investigators used the human tumor colony assay (HTCA), which he claims is the wrong *in vitro* endpoint. Within a given tumor only 0.01–0.001% of the cells are actively proliferating and capable of forming colonies, a prerequisite of the HTCA, says Nagourney. Thus, the older colony-forming assays only looked at the effect of a drug on a tiny fraction of the total cells, namely those that were rapidly dividing. Nagourney maintains that an effective therapeutic agent is not always the one that stops proliferation of cancer cells, as was assumed to be the case in previous efforts, but one that is effective in triggering the death of a tumor cell through apoptosis. In his words, 'The problem with cancer cells is not necessarily that they grow too much, but instead die too little'.

A new approach

Nagourney has developed the *Ex Vivo* Apoptotic (EVA) assay, which is used at Rational Therapeutics to evaluate the efficacy of various chemotherapy agents in inducing apoptosis of tumor cells. To utilize the EVA assay a physician sends a biopsy of a tumor to Rational Therapeutics under well-defined conditions that will facilitate the survival of the cells during transport. Once the biological sample arrives at the company, the cells of the tissue are dissociated using mechanical and enzymological techniques that are standard to cell culture, and the presence of a sufficient number of viable tumor cells for analysis is confirmed. If all goes well through this stage, the tumor cells are treated with a battery of antitumor drugs at a variety of different concentrations; drugs that are evaluated include those commonly used in cancer chemotherapy as well as experimental compounds.

After a 72 h incubation period with the selected drugs, the cells are examined microscopically by a cytologist to determine which drug regimen triggered the characteristic alterations in cell membrane integrity that are associated with apoptosis. As part of the standard procedure, an IC_{50} is determined for the ability of the various drugs to induce apoptosis. If a drug immediately kills the cell it is considered to be a nonspecific poison and of little interest. What is desired, says Nagourney, is a compound that will induce apoptosis after a 48–96 h exposure – 'It must induce the cell to decide to die'. The IC_{50} generated for an individual's tumor cells is then compared with data on similar types of tumors from Rational Therapeutics' database to determine if an individual tumor is more or less sensitive to a drug than its cohorts.

Using the EVA assay, Nagourney says that he is able to clearly identify the drugs to which the tumor cells are resistant and can also guide the physician to the appropriate drug or combination of drugs for an optimal therapeutic response. So far, there is no well-controlled, statistically valid study to show the superiority of Rational Therapeutics' approach to selecting optimal drugs for cancer chemotherapy. But Nagourney maintains that it is not at all unusual to discover that the optimal treatment regimen for the patients referred to him, based on the EVA assay, is a totally different drug or drug combination than would have been given if the standard protocol had been used and, in many

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cases, the standard protocol would have used a drug to which the patient's tumor cells were resistant. Nagourney claims that his approach can double the likelihood of a positive response to chemotherapy and diminish to zero the likelihood of a 'no response' by avoiding drugs that don't work. A meta-analysis of the new approach is in progress.

Teething problems

There is nothing proprietary about the EVA assay in the sense that the assay or any of its components have been patented, says Nagourney. 'But it is somewhat of an art,' he asserts, and the laboratory doing the assay must be very skilled in performing the visual analysis. The details of the delayed loss of membrane integrity on which the assay is based have been published[3], as well as the use of the technique as an end point to predict a therapeutic outcome[4].

So far, Rational Therapeutics' approach to the design of cancer chemotherapy remains highly controversial, and Nagourney's clients sometimes find it difficult to get their oncologists to accept the approach. Nagourney believes that this is because the oncology community concluded that 'this will never work' when the HTCA assays in the 1980s failed to predict accurately the outcome of cancer chemotherapy on a patient-by-patient basis. Some oncologists refuse to use the suggested protocol, as determined by the EVA assay, for chemotherapy. When this occurs, Nagourney can direct the patient to an oncologist who is willing to use the information he provides.

The EVA assay is useful for the discovery of new anticancer drugs as well as for finding the best uses for existing drugs, which Nagourney characterizes as 'dumb drugs that don't work very well'. The company is already collaborating with drug discovery scientists who wish to use the EVA assay to determine the best use for newly discovered anticancer agents. But 'the assay is not amenable to screening 40,000 compounds a year,' says Nagourney, 'so it will probably never be used as a primary screen'. He believes it will be most useful as a secondary assay after a compound has found promise as an antitumor agent. 'Once such a compound is identified,' continues Nagourney, 'we can tell you what type of cancer patient will benefit most from the new drug'.

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