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Perspectives and Commentaries

The Subrenal Capsule Assay (SRCA)

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(A COMMENT ON: Aho AJ, Maenpaa JU, Kangas L, Soderstrom KO, Auranen AA, Linna M. Subrenal capsule assay in human breast cancer. Response to cytostatic drug combinations and correlation to receptor status. *Eur J Cancer Clin Oncol* 1985, **21**, 1133–1140.)

THE SRCA was designed for testing developmental therapeutic agents against human tumor xenografts prepared from the solid malignancies [1]. Fundamental to the design were two considerations, (a) that solid tumors are composed of heterogeneous cell populations in terms of biosynthetic function, growth potential and drug sensitivity, and (b) that the complexity of epithelial/stromal relationship affects both tumor growth as well as drug sensitivities. By utilizing a tumor fragment for subrenal capsule implantation, cell membrane integrity, cell-to-cell contact, and the spatial relationship of the cell populations and tissues within the fragment are maintained permitting the measurement of tumor response to drug activity as a *net response* of multiple cell populations, both clonogenic and non-clonogenic. Thus, a degree of heterogeneity in the tissues comprising the xenograft is not only to be expected but is desirable if the fragment is a representative fraction of a patient's tumor. *Selection* of the tissue for subcapsular implantation in the SRCA is of prime importance.

In a recent study published in this journal, Aho, Maenpaa, Kangas *et al.* [2], observed that the histological features of subrenal capsule implanted human breast tumors on day 6 of the assay period suggested that under standardized conditions, i.e., with careful selection of tissues and supportive histology, the 6-day SRCA is a good method for studying the response of individual breast cancers to chemotherapy. This observation is of particular interest in view of a clinical study on the response of ovarian cancers to cytotoxic agents by two of the authors [3], in which the conclusion was made that the 6-day SRCA can be used to assess the response of ovarian cancers to chemotherapy, including multi-drug therapy, without routine histologic control. Although seemingly disparate, these conclusions only reflect the biosynthetic activities peculiar to the tissues being assayed, i.e., ovarian as contrasted to breast tissue. However, since the 6-day SRCA utilizes the normal immunocompetent mouse as host for human tumor xenografts, a somewhat unorthodox procedure, a comment concerning the histological features observed on day 6 of the assay period and the predictive quality of the 6-day SRCA needs to be made.

When non-necrotic, non-infected, fresh viable tumor tissue is implanted subcapsularly, one observes an increase in xenograft size that is directly related to the mitotic activity observed in the original tumor specimen [4]. Necrosis, whether inherent in the tumor or induced intentionally by a chemical agent, is quickly resorbed from the subcapsular site. Therefore, tumor size on day 6 is the resultant of cell division and resorption of nonviable cells. A decrease in the size of untreated control xenografts indicates implantation of necrotic tissue and an unevaluable assay.

Histology of the untreated xenograft on day 6, then, reflects (a) the quality of tissue implanted and (b) the resultant interaction of tumor cells and host tissues. It is reasonable to assume that host response to the xenograft is proportional to the amount of malignant tissue implanted and the persistence of such tissue to the end of the assay period, the former influencing the strength and the latter influencing persistence of the antigenic stimulus.

Assuming minimal trauma to the implant site, host cell infiltration of the xenograft becomes evident after day 3 increasing in intensity to day 6. Levi *et al.* [5] studied a number of solid human tumors of various histologic types in the SRCA and

found tumor microarchitecture to be intact and lymphocytic infiltration on day 4 to be minimal with fragments of fresh explants retaining proliferative and metabolic capacity for at least that period. Dumont *et al.* [6] found that a polymorphic inflammation which started on day 4 post-implantation was not artifactual to a tumor size parameter on day 6, and that an important polymorphic lymphohistiocytic granulomatous reaction occurred *after* that time. Reale [7] also found that tumor histologic architecture is preserved in explants out to day 6 in both athymic and immunocompetent mice and that infiltration of inflammatory cells does not significantly affect tumor size up to and including day 6. Interpretation of the source of inflammatory cells, particularly in human breast tumor xenografts, must be made with caution in view of the inflammatory reactions associated with certain breast malignancies. Also, inflammatory response to xenografts prepared from tumors of the oropharynx and colon may well be stimulated by bacterial contamination.

That suppression of an inflammatory response by an immunosuppressive chemotherapeutic agent may result in an overestimation of drug affect is not supported by the following observations. Thirty-one previously untreated human breast tumors were administered the same 50 mg/kg dose of cyclophosphamide on a QD1-5 regimen in the 6-day SRCA. Tumor responses as indicated by change (Δ) in tumor size are shown in the figure. The mean Δ tumor size values obtained with the drug treated tumors are connected (vertical lines) with their corresponding control values. It is clear that human breast tumors show, not only differences in growth potential and drug sensitivity to the SRCA, but that individual drug response is not directly related to the growth potential, e.g., tumors 1, 5 and 12 have similar control growth but each tumor exhibits a different sensitivity to cyclophosphamide and eight tumors were sufficiently resistant to cyclophosphamide to increase in size despite treatment.

Another histologic feature resulting from the persistence of viable tumor cells is the desmoplasia (excessive connective tissue) within and adjacent to the xenograft. Although many tumors both benign and malignant exhibit desmoplasia, the phenomenon is usually associated with invasive carcinomas of the breast, colon and prostate [8]. Desmoplasia is often so marked that it alone is responsible for the clinical manifestation of a tumor as a 'lump' [9]. The point being made is that assuming healthy malignant tissue is implanted, the histologic picture on day 6 can only reflect a quantitative host response to the presence of viable tumor tissue at the subcapsular site. Tumor cell synthesis of extracellular matrix components and the increased production of matrix components by host cells in response to the presence of tumor are phenomena associated with viable tumor cells.

Since chemotherapy is initiated early in the assay period, drug-sensitive tumor cells are necrotized early. As a result, the number of tumor cells surviving to day 6 is proportional to the sensitivity of the tumor to the test drug, and the reduction in the mass and viability of tumor tissue at the implant site is reflected by a proportional reduction of the tumor-mediated host responses at that site. One cannot assume that certain histological features evident on day 6 of the assay period are artifactual to a tumor size parameter or do not represent a quantitative response of the xenograft to chemotherapy. Conclusions drawn from observations made at a single time point may represent interpretation of a histological endpoint out of context.

Evaluation of tumor response by a 3-grade scale as suggested by Aho *et al.* [2] further sensitizes the assay. However, in malignancies such as primary breast tumors, where the epithelial/stromal ratio favors stroma and the excursion of tumor sizes between drug sensitive and drug resistant tumors may be more limited, a 3-grade scale should have merit. In the reference study, the authors suggest that the greater sensitivity of PR- and ER- tumors to cytostatic drug combinations might be due to the relatively higher mitotic activity often seen in such anaplastic tumors. In our own studies, when growth rates and drug response profiles of 75 ovarian and 71 lung tumors, tested in the 6-day SRCA, were sampled at random and analyzed to determine whether the SRCA was adequately sensitive to detect a difference in drug sensitivity between fast growing and slow growing tumors, as observed clinically, we found that both the response rates and the degree of tumor regression were greater in fast growing tumors [12], supporting Aho's suggestive evidence on breast tumors.

There is no question that the 6-day SRCA is only as good as the tissue implanted. Assistance of the surgeon and/or pathologist in the selection of tumor tissue is a significant advantage. As an additional histological quality control at the laboratory level, we would recommend a modification of the procedure proposed by Levi *et al.* [5], to randomly select five tumor fragments at the beginning and five at the end of the implantation period, and prepare a squash or blot followed by a rapid H&E or vital stain. Tumor fragments are acceptable for assay implantation when 7 of 10 (70%) of the fragments contain tumor cells. With six to eight animals per test group and careful randomization, differences in tumor response to active and inactive drugs will be statistically significant.

In the final analysis, the value and acceptance of

Table 1.

Investigator and institution	Type of malignancies	Evaluable Assay Rate
R. Favre *et al.* Inst. J. Paoli I. Calmettes, Marseille, France	Various	88%
T. Griffin *et al.* Univ. Mass. Medical Center, Worcester, MA (U.S.A.)	Various	86%
J. Stratton *et al.* Univ. of California Medical Center at Irvine, CA (U.S.A.)	Gynecologic	89%
J. Maenpaa, Univ. of Turku, Turku, Finland	Gynecologic	97%

any predictive test is primarily based upon (a) an acceptable rate of evaluable tests, (b) that test results are provided to the oncologist within a clinically acceptable time frame, (c) its ability to identify developmental agents that will have clinical efficacy, and (d) to direct chemotherapy of the individual cancer patient. When used to screen cancer patient populations for sensitivity to known clinically active drugs, there has been excellent concordance of response rates obtained in the 6-day SRCA and that observed clinically for breast cancer using single agents [10] and drug combinations [2], for head and neck cancer [11], malignant melanomas [6] and ovarian tumors [3].

Effectiveness of the 6-day SRCA as a predictive test for individualizing cancer chemotherapy is also borne out by the 398 assay/clinical correlations obtained in both retrospective and prospective clinical studies on 301 patients at four institutions (see Table 1). The overall evaluable assay rate obtained with approx. 1400 fresh surgical explants of solid tumors was 90%. The incidence of false negatives in all of the reviewed trials was 3%. The assay predicted better for clinical sensitivity (91%) than for resistance (73%). This difference is due to the false positives (19%) resulting, e.g., from differences in the tumor burden treated in the assay as compared with that treated in the patient, ability of the drug to reach therapeutic concentrations in the patient's tumor, or the emergence of drug resistant cell populations during chronic therapy.

One can conclude that although all mechanisms operative at the subcapsular implant site have not been completely elucidated, the assay/clinical correlations suggest that host cellular reactions are not artifactual to a tumor size parameter for evaluating tumor response and support the predictive quality of the 6-day SRCA. The urgent need for a predictive assay, SRCA or otherwise, cannot be overemphasized. With the increasing number of 'effective' chemotherapeutic agents entering the clinic, and with the spectre of pleotrophic drug cross-resistance, it is time that individual patient treatment be guided by considerations more specific than response rates.

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