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Clinical Implications of Tumor Heterogeneity *

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Clinical oncologists have long recognized the great variability in the cellular morphology, natural history, and response to therapy of human tumors. Recent progress in molecular biology, biological chemistry, immunology, and other disciplines has now provided scientists with an array of new technologies that has allowed the study of neoplastic disease to go beyond morphologic and clinical description to examination of the malignant state at the cellular and molecular level. The availability of monoclonal antibodies, DNA hybridization techniques, hormone receptor assays, human tumor stem cell assays, and other methods now enables investigators to reveal, in greater detail than ever before, the great phenotypic and genotypic diversity present in most primary and metastatic tumors. Much of the information obtained thus far has been largely descriptive, cataloging the diversity in karyotypes, immune phenotypes, metastatic potential, drug sensitivity, and other cellular characteristics that commonly occurs in tumors that are seemingly identical morphologically.

The origin of tumor heterogeneity is less clearly understood, although the genetic instability inherent in the malignant state appears to be an important element in its generation and maintenance [1, 2]. Tumor heterogeneity is a dynamic process, and changes in the composition of a neoplasm occur over a period of time in response to environmental selection pressures generated within the tumor (e.g., competition for nutrients), by the host's immune defense or imposed by the treating oncologist [3–5]. Indeed, tumors may be viewed as continually evolving within the host, with the treatment-resistant phenotype representing survival of the "fittest" malignant cells. This process is not random and uncontrolled, however, but is regulated in some way by interactions among the cellular subpopulations comprising the tumor such that rapid clonal diversification occurs under conditions of limited cellular diversity, thus ensuring the continued survival of the tumor in the face of varied therapeutic attempts.

Of considerable importance to clinical oncologists is the fact that much of this heterogeneity may be generated prior to clinical detection of the tumor. Even a 1-cm tumor mass contains at least a billion tumor cells, and the number of mitoses that a single cell must undergo to reach this volume will depend upon both the rate of cell growth and the rate of cell loss from the tumor mass. With the potential for genetic mutation to occur with each mitosis, it is not surprising that phenotypic diversity commonly occurs even in early-stage tumors.

An enormous challenge is thus presented to clinical oncologists from the perspective of assessing the "clinical relevance" of tumor heterogeneity and because of the need to develop new treatment strategies able to effectively eradicate multiple tumor subpopulations.

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The clinical importance of identifying tumor subpopulations is directly related to the impact such knowledge has on determining prognosis and making management decisions. Among the large-cell non-Hodgkin lymphomas, for example, histologic subtyping was initially reported to have a significant impact on treatment outcome and prognosis [6]. Patients with the blastic and pleomorphic pyroninophilic subtypes had a significantly worse prognosis than patients with the other histologic subtypes described. However, with the development of more aggressive and effective chemotherapy treatment regimens, differences in outcome for these histologic subtypes have disappeared [7]. Similarly, the use of monoclonal antibodies for immune phenotyping of non-Hodgkin's lymphomas has revealed a diversity not appreciated by standard morphologic analysis alone [8]. However, the therapeutic ramifications of detecting a particular immune phenotype on lymphoma cells are as yet unknown. Indeed, the overall effectiveness of combination chemotherapy in the treatment of diffuse large-cell lymphomas suggests that recognition of a particular immune phenotype in these diseases may not provide information of any practical importance. In other malignancies, however, such as childhood acute lymphoblastic leukemia (ALL), immune phenotype may well be an important determinant of prognosis and, to some extent, therapy [9], although further advances in ALL treatment are likely to diminish the importance of this prognostic factor as well. The clinical relevance of cellular heterogeneity to the prognosis and management of other tumors is presently unknown but can be assessed through well-designed clinical trials stratified prospectively for phenotypic variables. Clearly, the importance of phenotypic heterogeneity for some characteristics will diminish as therapy continues to improve.

The demonstration that tumor heterogeneity is a common phenomenon could easily create an air of pessimism among clinical oncologists. After all, it appears that tumors are infinitely adaptable, possessing the ability to metastasize widely prior to clinical detection and rapidly evolve new antigenic properties, hormone receptor levels, and patterns of drug resistance. Tumors, it seems, are always one step ahead of the treating physician. The alternative view, however, is that the recognition and understanding of tumor cell heterogeneity may in fact provide the foundation upon which successful new treatment strategies can be developed. One area of recent progress, for example, is the development of a new in vitro model for growing malignant cells as they exist in vivo. Traditional in vitro models rely on the growth of tumor cells in monolayer or suspension culture, conditions which rarely, if ever, re-create the growth of tumors in vivo. By contrast, multicellular spheroids approximate many characteristics of in vivo tumor growth, including three-dimensional intercellular contact, ranges in pH, oxygen tension, nutrient levels, and the ability to be grown in culture for weeks without trypsinization [10]. Yet spheroids can be grown under carefully controlled environmental conditions and may therefore provide a unique in vitro model of tumor cell heterogeneity. Indeed, this model has already been used successfully to explore the interactions between drug-sensitive and drug-resistant brain tumor cells [11]. Multicellular spheroids may well serve as a model of cellular heterogeneity of potential utility in drug sensitivity testing and screening for new agents. With such a system, new drugs could be selected for clinical trial on the basis of their activity in a screening system that more closely resembles tumor growth in vivo than those systems currently in use. The human tumor stem cell assay [12] and the development of drug-resistant human tumor cell lines offer other advantages over the traditional drug-screening systems and are currently being evaluated as experimental systems that could be employed in a more rational approach to screening for potential new cytotoxic agents.

While new drug development continues to be an important area of research, other more novel approaches to cancer treatment are being pursued in an effort to circumvent the problems created by tumor heterogeneity. Activated macrophages, for example, have been shown to selectively destroy tumor cells, while leaving normal cells intact [13]. In vitro, these cells are able to recognize and destroy many types of tumor cells, regardless of such cellular variables as metastatic

potential and drug sensitivity. Lymphokine-activated killer (LAK) cells also exhibit the desirable property of nonspecifically killing tumor cells while leaving normal cells intact, a fact which suggests that they recognize a feature common to tumor cells that is not expressed by normal cells [14]. The possibility thus exists that in vivo activation of tumoricidal macrophages or LAK cells may be a useful therapeutic adjunct to conventional cytotoxic therapy. Ongoing clinical trials of LAK cells plus interleukin 2 may soon shed some light on this question.

The use of monoclonal antibodies to deliver radioisotopes or cellular toxins directly to the vicinity of a tumor mass may provide a more efficient system for the application of nonspecific cellular poisons in a tumor-specific way. The use of radioisotope antibody conjugates is particularly attractive since the energy emitted by the isotope may be sufficient to destroy those cells in the vicinity of the conjugate, even though antigenic heterogeneity may prevent the binding of the monoclonal antibody to each individual tumor cell.

Another novel approach to circumventing the problem of tumor heterogeneity lies in the use of agents capable of inducing tumor cell differentiation to a more "benign" phenotype. It has been postulated that drugs such as the polar solvents *N, N*-dimethylformamide and *N*-methylformamide could induce tumor cell maturation, limit the continued generation of tumor subpopulations, and thereby produce a more homogeneous tumor more likely to be eradicated with conventional treatments [15]. Interestingly, these agents have been shown to enhance the cytotoxic effects of alkylating agents [16] and ionizing radiation [17] in experimental systems. Clinical trials are currently in progress, and the results are awaited with great anticipation.

Though therapeutic approaches such as those discussed above hold great promise for the future, it is probable that conventional cytotoxic chemotherapy will continue to play a major role in cancer treatment. As such, a better understanding of the mechanisms by which drug resistance develops will enable the development of treatment stratagems to circumvent it. For some drugs, the biochemical mechanisms of resistance are well understood, and this knowledge has been applied to the development of drug analogs able to circumvent the resistant state. In the case of methotrexate (MTX), cellular resistance can develop owing to impaired membrane transport, increased content of dihydrofolate reductase (DHFR), altered affinity of DHFR, or impaired polyglutamylation [18]. There now exist methotrexate analogs able to overcome most of these potential mechanisms of resistance. Lipophilic diaminopyrimidine antifolates have been developed that are cytotoxic to transport-deficient MTX-resistant cells [19]; 2-amino-4-hydroxy-quinazoline antifolates have been synthesized that are able to inhibit thymidylate synthase directly, independently of DHFR content [20]; and a new trimethoxy quinazoline derivative of MTX – trimetrexate – which does not undergo polyglutamylation and is not cross-resistant with transport-deficient cells is currently undergoing clinical testing [21]. Thus, there now exist MTX analogs capable of overcoming essentially all known mechanisms of antifolate resistance. Combination chemotherapy with multiple antifolates has been effective in overcoming MTX resistance in vitro [22], and it is tempting to speculate that clinical chemotherapy with an antifolate combination might be effective as well. Indeed, recently reported studies in tumor-bearing animals indicate that the sequential use of MTX followed by trimetrexate is more effective than treatment with optimal doses of either drug alone [23].

Another strategy to overcome drug resistance that has received widespread attention is the use of calcium channel blockers and calmodulin inhibitors to enhance cellular sensitivity to anthracyclines, vinca alkaloids, and other drugs. Verapamil [24], trifluoperazine [25], and related drugs can successfully overcome doxorubicin resistance both in model systems and in drug-resistant human tumor cells [26]. Clinical studies are currently in progress to determine whether these drugs can be successfully used to circumvent resistance in patients with refractory tumors.

Understanding the heterogeneous nature of the malignant state serves only to emphasize the importance of one of the long-standing rubrics of the fight against cancer – early

detection. Indeed, early detection and early application of effective therapy remain two of the most effective methods of limiting tumor cell heterogeneity and circumventing drug resistance. The mathematical model of drug resistance developed by Goldie and Coldman [27] suggests that resistance can develop rapidly, within only a few cell divisions; that alternation of non-cross-resistant regimens may be beneficial; and that chemotherapy must be given in high cytotoxic doses, lest its mutagenic potential actually contribute to the development of drug resistance. The application of intensive combination chemotherapy immediately following diagnosis and even prior to definitive local therapy may be the most effective method of eradicating micrometastases and producing long-term disease-free survival. Preoperative chemotherapy appears promising in head and neck cancer [28], osteogenic sarcoma [29], and even non-small-cell lung cancer [30], though many more years of follow-up are necessary before the final results are obtained. Nevertheless, these initial clinical trials should serve as the basis for the continued evaluation of primary chemotherapy in the management of solid tumors.

The time has come for clinical oncologists to look carefully at the evolving concepts of tumor biology, for from these concepts will come the clinical trials and treatment strategies of the future. The last decade has seen a tremendous increase in our knowledge of the extraordinary diversity of the malignant state. Armed with this knowledge, clinical oncologists can look forward to continued success in the development and application of new therapies likely to rapidly advance the state of the art of cancer treatment.

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