Tumor heterogeneity: biological implications and therapeutic consequences

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Summary

It is now appreciated that cancers can be composed of multiple clonal subpopulations of cancer cells which differ among themselves in many properties, including karyotype, growth rate, ability to metastasize, immunological characteristics, production and expression of markers, and sensitivity to therapeutic modalities. Such tumor heterogeneity has been demonstrated in a wide variety of animal tumors of differing etiology, tissue and cellular origin, and species. It has been shown in autochthonous, as well as transplanted, tumors. Similar results have been reported for human cancers, although much of the evidence that heterogeneity of human cancers, also reflects, at least in part, the existence of clonal subpopulations, is still indirect. Heterogeneity is not a unique property of malignancy. Preneoplastic tumors, as well as normal tissues, are also composed of cellular subpopulations.

Proposed mechanisms for the origin of tumor heterogeneity include coalescence of multiple loci of cancer clones and the generation of diverse subpopulations from a single clone. This latter process could be due to genetic errors arising from classical genetic mechanisms or to the production of cellular variants as in normal tissue differentiation. Indeed, certain tumor subpopulations have been shown to produce variants at high frequency. In some cases this frequency can be modified by environmental circumstances. Nontumor cells may also contribute to production of cancer cell variants, perhaps, in the case of infiltrating phagocytic cells, by producing mutagens or by somatic hybridization with cancer cells. Production of tumor cell variants is a dynamic process which can occur at any time.

Although tumors are mixed populations of cells, knowledge of the characteristics of individual components is not sufficient to predict the behavior of the whole. Individual cancer subpopulations can interact to affect each other’s growth, immunogenicity, ability to metastasize, sensitivity to drugs, and clonal stability. The existence of multiple, interactive subpopulations provides a basis for the well-known phenomenon of ‘tumor progression’ in which tumors undergo qualitative changes in characteristics over the course of time. Selection of subpopulations better able to survive changing environmental circumstances allows for such changes as autonomy in regard to endogenous growth regulation, more ‘malignant’ behavior, and loss of response to therapy. Tumor subpopulation interactions may play a regulatory role in this process.

Tumor heterogeneity has obvious consequences to the design of effective therapy. It provides one rationale for combination therapies and suggests that initial treatment should be early and comprehensive. The continuing emergence of new clones suggests that treatment which is unsuccessful at one point might be
effective later. Assays to predict effective therapy for individual patients need to address the multiplicity of tumor subpopulations and the ability of these subpopulations to influence each other. Subpopulation interactions may also be useful in therapy design, as may be efforts to control the extent of tumor heterogeneity by agents which effect cellular differentiation. Thus, tumor heterogeneity presents both problems and, perhaps, new solutions for control of cancer.

**Introduction**

The idea that tumors are not uniform populations of 'cancer cells' has gained new strength in the past few years. Attention is now focused on the many ways by which cancers differ and on the basis for these differences. This has led to the rediscovery of concepts of tumor biology which were known to cancer researchers in the past but which had become lost during the euphoria of the revolution in molecular biology. The purposes of this review are to document the increasing evidence for one such concept – tumor heterogeneity – and to speculate on its implications to tumor biology and consequences to cancer therapy.

**Definition of tumor heterogeneity**

Tumors are 'heterogeneous' in several ways. There is the heterogeneity among cancers in different individuals who nominally have the same type of disease. It is this heterogeneity which fuels the search for prognostic indicators and for methods to individualize therapy. A second type of heterogeneity is that seen within the same patient over the course of time. The biological, as well as the clinical, characteristics of an 'early', preinvasive tumor are not the same as those exhibited by the same cancer when it has disseminated. This type of heterogeneity is acknowledged by Fould's concept of 'progression' (1).

Heterogeneity is also seen within a single tumor at any one time. Histological examination of tumor samples often reveals considerable differences in the morphology of cancer cells in different areas of the same lesion. Host infiltrating and connective tissue are not evenly distributed. Areas of necrosis may be present. Depending upon tumor size, marked disturbances in vasculature can occur, leading to focal differences in oxygen tension, pH, substrate supply, and waste drainage (2). Related in part to this structural heterogeneity is heterogeneity in growth compartments. The cells within a tumor may be cycling or noncycling, quiescent or reproductively dead (3). If cycling, they may be at any stage in the cycle. Insofar as stage of cell cycle may influence cellular properties such as membrane biochemistry (4, 5), antigen expression (6–8), sensitivity to immune killing (9, 10), drug cytotoxicity (11), and ability to metastasize (12, 13), tumors will be heterogeneous in regard to those properties.

The type of heterogeneity which has received the most attention, and which is the subject of this review, is that due to the simultaneous existence of multiple clonal subpopulations within the same tumor. It is well to remember that such subpopulations are individually subject to all the other types of heterogeneity described above: as will be described, new subpopulations can arise during neoplastic progression. Furthermore, depending upon local conditions, structural and cell–cycle heterogeneity will be present within, as well as among, subpopulations. In addition, subpopulation heterogeneity imposes additional structural heterogeneity on the tumor as a whole. Cells in individual subpopulations may be located in distinct areas, or zones, of a tumor, rather than comingling (14–16). The zonal distribution of tumor subpopulations needs to be taken into account in devising methods of sampling tumors for various types of analysis. Investigators who serially transplant tumors in vivo with pieces of tumor, rather than cell suspensions, in reality may be transplanting only certain subpopulations.

**Heterogeneity of experimental tumors**

The coexistence of multiple subpopulations of tumor cells within single neoplasms has been re-