Cancer Metastasis Reviews 2:5-23 (1983)

Tumor heterogeneity: biological implications and therapeutic consequences

Gloria H. Heppner and Bonnie E. Miller
Michigan Cancer Foundation, Detroit, MI 48201, USA

Keywords: tumor heterogeneity, clonal instability, tumor progression, subpopulation interactions, therapy

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 foci 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

Address for reprints: Dr. Gloria H. Heppner, Department of Immunology, Michigan Cancer Foundation, 110 E. Warren Avenue, Detroit, MI 48201, USA

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-

peatedly demonstrated in animal tumors of diverse etiology and histological type. These include melanoma (17–19) lymphoma–leukemia (20, 21), sarcoma (14, 22–26), and carcinoma (27–35). Heterogeneity in tumors induced by chemical agents (24, 32), physical agents (19, 25, 26), steroids (28), or viruses (20–22, 27, 30, 33–35) has been described. Long-term passaged tumors (18, 23, 24, 31), tumors of recent origin (19, 25, 26), and autochthonous tumors (20, 30) have been the source of multiple subpopulations. At this time it appears that no class of neoplasm is excluded from being heterogeneous, but quantitative differences among classes may be revealed by further experience.

Tumor heterogeneity is manifested by a variety of phenotypic differences. Differences in cellular morphology (30) and tumor histopathology (21, 29, 36, 37), as well as differences in growth rate, both in vivo and in vitro, have been seen (17, 19, 30, 31, 37). Tumor subpopulations can differ in expression or production of 'markers' of differentiation, including appropriate pigment (16, 17), receptors (38), cell products (21), and specialized biosynthetic enzymes (28). Phenotypic diversity has also been reported for immunological characteristics, including antigen expression, immunogenicity, and sensitivity to immune attack (14, 20, 30, 34, 39–44). (Immunological heterogeneity has been reviewed in depth elsewhere in this series (45).) Perhaps the most significant phenotype by which tumor subpopulations can differ is ability to metastasize. Following the lead of the classic experiment by Fidler and Kripke with the B16 mouse melanoma (18), the existence of tumor subpopulations that vary in ability to metastasize has been demonstrated in several experimental systems, including a recently isolated u.v.-induced melanoma (19), a variety of sarcomas (23, 25, 26), and mouse mammary tumors (31, 46, 47).

Primary tumors contain subpopulations that can metastasize to specific organ sites at high, medium, or low frequency, relative to the parent tumor (23). On the other hand, subpopulations that are unable to metastasize (at least by themselves), and may even be unable to produce tumors except at high innocula and after prolonged latency periods (30, 37, 48, 49), can be isolated from highly tumorigenic parent neoplasms. As will be discussed below, the simultaneous existence within a single tumor of subpopulations that differ, when tested independently, in degree of tumorigenicity suggests that within the parent tumor there are interactive mechanisms among the subpopulations that regulate growth and dissemination.

In addition to differences among tumor cell subpopulations, nonmalignant tissue within neoplasms may also be heterogeneous. Recent results from our laboratory suggest that normal cell heterogeneity may be related to tumor cell heterogeneity. Infiltrating lymphocytes have been isolated from solid mammary tumors produced by a series of cell lines which were originally derived from a single strain BALB/cfC$_3$H mouse mammary tumor. Not only did the percentage of lymphocytes isolated vary among the lines, but the type of lymphocyte also differed. In particular, the relative proportion of T cells belonging to the helper class versus those identified as members of the killer-suppressor class was characteristic for different tumor subpopulations (50). Tumor-infiltrating cells independently isolated from two different subpopulations growing on the same mice belonged to the T cell type characteristic for the individual subpopulations. Thus, the type of T lymphocyte response was a characteristic of the tumor, not the host, and was associated with specific tumor cell subpopulations. Whether tumor cell heterogeneity similarly influences other host components of tumors remains to be determined.

The wide range of phenotypic differences among tumor cell subpopulations suggests the existence of genotypic differences. Indeed, numerous investigators have described karyotypic differences (22, 30, 37, 51–55), as well as the presence of different marker chromosomes in different tumor subpopulations (37, 56). Using murine mammary tumor virus (MuMTV) DNA as a probe, cellular heterogeneity in the location and copy number of a specific gene has been demonstrated in strain GR mouse mammary tumors (33, 35). This is in accordance with the heterogeneity in expression of MuMTV-coded antigens within individual mammary tumors (34). Studies on the differential re-

sponse of BALB/cfC$_3$H mammary tumor subpopulations to inducers of MuMTV gene expression suggest that differences in regulation of MuMTV genes also correlate with tumor subpopulation heterogeneity (57).

Heterogeneity of human cancers

There is considerable indirect, and increasing direct, evidence that human cancers, like their animal counterparts, are composed of heterogeneous subpopulations. Heterogeneity in histological pattern may be seen in multiple samples of breast carcinoma (58, 59) and in small cell anaplastic carcinoma of the lung (60). Histological and ultrastructural heterogeneity of tumor cells from bronchial carcinoid has been described (61). Intratumor heterogeneity in tumor cell DNA content has been observed in colon carcinoma (62) and small cell carcinoma of the lung (63). Expression of tumor-associated antigens has been shown to be nonuniform among cells from single neoplasms, such as osteosarcoma (64), and pancreatic (65), and breast carcinoma (66). Other markers of tumor cell differentiation have likewise been shown to be distributed heterogeneously within tumors, for example, B_2-microglobulin (67) and estrogen receptors (68–70) in breast cancer. Tumor cell heterogeneity for calcitonin has been described in virulent medullary carcinoma (71). This is especially interesting in that it was shown that heterogeneity for calcitonin staining was seen in medullary carcinomas with a high likelihood of metastatic spread, whereas uniform staining was seen in tumors with a small chance of recurrence.

Additional evidence that human cancers contain tumor cell subpopulations comes from comparison between primary tumors and metastases. Here again one may see divergence in histological type (59). Differences in levels of histaminase and L-DOPA decarboxylase have been reported between primary small cell carcinoma of the lung and hepatic metastases (72). Different hepatic metastases from the same patient likewise vary in L-DOPA decarboxylase activity. Differences in sensitivity in vitro to antineoplastic drugs between cells from primary ovarian carcinomas and their metastases have also been seen (73). Furthermore, estrogen receptor content can vary between primary breast cancers and their metastases and among multiple metastases of the same patient (70).

As with animal tumors, formal proof of the existence of tumor subpopulations requires their isolation and characterization. This has now been accomplished with a growing list of human tumors. Tumor lines that differ in drug sensitivity (74, 75), antigenicity (76, 77), or tumorigenicity in nude mice (78) have been isolated from single melanomas, both from primary lesions (74, 75, 78) and multiple metastases of the same patient (77, 78). Tumor subpopulations have also been isolated from primary human colon carcinomas (79, 80). Certain of these subpopulations differ in karyotype (80), in vitro growth properties (79, 80) tumorigenicity (79) and histology of tumors in nude mice (79–80). Similar isolations of tumor subpopulations have also been reported for lung (81), ovarian (82), and pancreatic (83) cancer.

Isolations of tumor subpopulations from human cancers have frequently been accomplished using cell cultures which had been maintained and passaged in vitro for fairly long periods of time prior to cloning. Only rarely have the subpopulations been obtained directly from the patient (77, 82). This raises the possibility that the production of heterogeneous variants is a consequence of the in vitro environment and occurs sometime after removal of the tumor from the patient. In this regard the elegant study of Shapiro et al. (84) needs emphasis. These investigators karyotyped tumor cells from fresh samples of human gliomas within six to 72 h after surgery. An array of unique karyotypes was found in each tumor. Simultaneously, dissociated tumor cells were cloned by dilution plating and the clones were karyotyped. By matching karyotypes of the clones with those in the fresh sample, it was possible to show that the clones were present at the time of resection. Each of eight gliomas was found in this way to have from three to 21 subpopulations – a minimal estimate since different subpopulations can have similar karyotypes. Different clones from the same tumor differed in morphology and growth kinetics. Antigenic heterogeneity has also been

reported in clones derived from a single human glioma (84).

The work of Shapiro et al. (81), as well as work done with animal tumors (18, 30), suggests that heterogeneity is not induced by culture in vitro. On the other hand, it is often assumed that long-term cell lines are not heterogeneous, or minimally so, due to selection in vitro. That this is not so is shown by the ability of investigators to isolate subpopulations from lines such as murine L1210 (40, 41) and human tumor lines, including HT29 colon carcinoma (86), MOLT-3 malignant T-lymphoblasts (87), MCF-7 breast carcinoma (88), and other established lines (76, 80, 81, 83, 84).

Origin of tumor heterogeneity

A point of confusion in understanding tumor heterogeneity is reconciling its existence with the large body of evidence pointing to a single cell origin for many, if not most, neoplasms (89). Strong as this evidence is, it must be remembered that it is not universal. Some tumors, such as 'venereal' warts in man (90) and fibrosarcoma induced by relatively high doses (91) of methylcholanthrene in mice have been shown to arise from more than one clone (92). Furthermore, some human cancers are characterized by numerous foci of neoplastic change. Multiple lesions of hyperplastic, in situ, and intraductal neoplasia can often be demonstrated in breasts of women presenting with invasive breast carcinoma (59). Thus, a developing malignancy could incorporate elements from other lesions, and hence become 'heterogeneous'.

Even if all cancers were truly of single cell origin, the opportunity for heterogeneity to develop occurs as soon as that single cell divides. As will be discussed, both structural and regulatory alterations in genetic function may contribute to cellular variation. After all, most multicellular organisms begin as single cells. Even among cells from grossly homogeneous tissues, biochemical and functional heterogeneity is apparent. The heterogeneity among hemopoietic cells, ultimately derived from clonal stem cells (93), and the multiple cell types within the lymphocyte family (94) are obvious examples. Even quite similar cells, such as thymocytes (87) or mammary epithelial cells (95) are heterogeneous in regard to enzymatic activity or antigen expression. Griffin et al. (96) have demonstrated that normal cells can be cloned into heterogeneous subpopulations; different clones of genital skin fibroblasts display a wide range of activity of 5α-reductase, the enzyme that catalyzes the conversion of testosterone to dihydrotestosterone.

If normal tissues exhibit cellular heterogeneity, it is not surprising that minimally transformed or preneoplastic tissues would do so also. Heterogeneity in expression of a battery of marker enzymes within foci of hyperplastic, preneoplastic hepatocytes has been demonstrated at the earliest time of recognition of such lesions (97). Intranodule heterogeneity in expression of MuMTV antigens was seen in mammary hyperplastic alveolar nodules of MuMTV-infected mice (98). Similarly, chromosomal analysis of tumors produced by subcutaneous implantation of $C_3H/10T\frac{1}{2}$ cells attached to plastic suggests that they arose from minor subpopulations within the original culture (99), indicating a heterogeneity within that line in regard to induction of tumorigenicity. That such heterogeneity can have a genetic basis was shown for susceptibility to ultraviolet light-induced transformation by cloning differentially susceptible variants from BALB/3T3 cells (100). Thus, cellular heterogeneity is present before, as well as after tumor production, and is itself a factor in tumorigenesis. Clearly such heterogeneity is not unique to cancers, and tumor heterogeneity does not *necessarily* require any special explanation.

Numerous mechanisms have been proposed for the production of diverse subpopulations within a developing tumor. The most pervasive ideas are those of Nowell (55) who theorized that concomitant with the initiation of neoplasia within a single cell is the acquisition of genetic instability beyond that seen in normal cells and not due only to loss of growth restraints. Nowell cited studies showing a higher frequency of genetic errors in neoplastic than in normal cells and futher suggested that genetic instability becomes greater as a neoplasm evolves. Direct evidence for this latter hypothesis has recently been presented by Cifone and

Fidler (101) who showed that the rate of spontaneous mutation is higher in fibrosarcoma cells of a metastatic clone than in similar cells of nonmetastatic subpopulations. The type of genetic errors in neoplastic cells could include mutations in structural genes, mutations in regulatory genes, major chromosomal rearrangements and losses, gene amplification, and subtle rearrangements in specific gene positions resulting in alterations in gene regulation (55, 102). Evidence for the role of genomic rearrangements in contributing to variant production during tumor development (and being related to the multiple steps observed in tumorigenesis) has recently been described by Smith and Sager (103). These rearrangements can be nonrandom, structured alterations which reproduce precisely from experiment to experiment (102). Nowell also indicated a special role for viral oncogenes in this process, suggesting that variability in number and place of insertion sites could result in position effects on gene regulation. Subpopulations of cells with such differences in viral gene integration have been described in mouse leukemias and mammary tumors (35, 104) and, in the latter case, related to specific clones within a single tumor (35).

Other mechanisms for the generation of tumor heterogeneity exist which do not necessarily require structural alterations in the genome. Pierce (29) was one of the first investigators to suggest that neoplastic stem cells could give rise to variants through a process resembling normal tissue differentiation. Single cells isolated from a murine teratocarcinoma differentiated in vivo into a wide variety of tissues, representing all three germ layers. The progeny of the malignant stem cells were nonmalignant. Pierce stated that teratocarcinoma was a 'caricature of embryogenesis'.

That teratocarcinoma is not a special case is shown by similar results with tumors of diverse origin, including a chemically induced rat squamous cell carcinoma (29), two types of chemically induced rat mammary adenocarcinomas (105, 106), a virus-induced mouse mammary adenocarcinoma (37), a chemically induced rat neurotumor (107), and the MOPC-315 murine myeloma line (108). Some interesting differences among these various systems are apparent. The teratocarcinoma and squamous cell carcinoma stem cells differentiate to benign cells, whereas the others give rise to neoplastic variants. The rat mammary carcinoma tumors show a differentiation of epithelial-like cells to spindle-shaped, fibroblast-appearing cells, whereas the mouse mammary carcinoma, neurotumor, and myeloma have a broader potential, giving rise to a spectrum of variants. Some of the variants produced by the mouse mammary tumor are also variant producers. Interestingly, although this mammary tumor produces variants at high frequency only in vivo, the variant-producing cells produced by it can produce further variants in vitro. The rat mammary tumor lines and the neurotumor produce variants in vitro, although the frequency of variant production has been shown to vary from clone to clone in at least one of the rat mammary tumors (106). Taken in the aggregate these data suggest that the degree of differentiation potential of a neoplastic stem cell is a reflection of the potential of its normal counterpart. The frequency of differentiation, however, may reflect additional genetic and environmental factors.

The role of environmental versus genetic factors in the generation of tumor heterogeneity is a complex problem. Several investigators have assumed that the high frequency of variant production argues against somatic mutation as a primary mechanism (106, 109). However, quantitation of variant production, which can involve many phenotypic changes and, undoubtedly, numerous pleiotropic effects, is not as straight forward as quantitation of mutation frequency. Expectation of mutation rates in mammalian cells are, in the main, based on experiments with normal cells. As already mentioned, mutation frequency in tumorigenic and malignant cells can be considerably higher (55, 101). Furthermore, it is usually assumed that the 'environment' in which variant production is occuring is free of mutagens. That this may not be the case is suggested by recent experiments performed by Scott Loveless in our laboratory (110). Starting with an observation that human monocytes can increase the mutation rate of *Salmonella typhimurium* in the Ames assay (111), Loveless isolated macrophages from a series of mouse mammary tumors and similarly assayed their mutagenic activ-

ity. Macrophages isolated from tumors capable of spontaneous metastasis increased the mutation rate over ten times above background. Nonmetastatic tumors were less mutagenic. Results of Weitzman and Stossel (112) indicate that the mutagenic activity of phagocytes is related to their production of oxygen radicals. Whatever the mechanism, it would seem that the conjunction of host infiltrating cells capable of inducing mutation with tumor cells known to be genetically unstable might result in mutation rates considerably greater than what has been thought likely. Reports on the necessity, in some systems, of in vivo passage to induce variant production may indicate a role for tumor-associated host cells in the generation of heterogeneity (37, 109, 113).

Another role for normal host cells in the generation of tumor variants may be through the process of somatic cell hybridization (59). DeBaetselier et al. (114) have shown that hybridization of nonmetastasizing murine plasmacytoma cells with normal spleen B lymphocytes results in the production of variants capable of spontaneous metastasis. Furthermore, these variants exhibit distinct organ specific patterns of spread, growing preferentially in liver or spleen. Other investigators have shown that fusion between tumor and host cells can occur in vivo (115–117). One can imagine that tumor cell variants could be generated by such an event followed by genomic rearrangements, unequal distribution of chromosomes, or chromosome loss at division. This sort of mechanism may also be responsible for the observations of 'carcinogenic tumors', in which transplantation of tumor cells results in tumor formation by host cells (118–120). A most interesting example of this phenomenon is that of Kerbel and associates (119, 120) who showed that injection of any of five, independent strain A mouse tumors into DBA/2 mice results in the production of DBA tumors, at a 100% frequency, after a very short time. Interestingly, the new DBA tumors are very similar to each other and are always highly metastatic. Clearly, however, mechanisms other than somatic cell fusion may be responsible for these observations.

This brief review of the origin and mechanisms of tumor heterogeneity reveals two major points: heterogeneity is not a property exclusive to tumors, but is seen in normal tissues as well, and the potential mechanisms for generation of tumor heterogeneity are many and interrelated. Are any of the mechanisms unique to tumors, or are the differences in the origin of tumor versus normal tissue heterogeneity matters of differences in frequency or control? Somatic cell fusion can occur between normal cells in vivo (117). Furthermore, recent discoveries in molecular genetics, such as 'jumping genes' and generalized RNA to DNA pathways, point to a flexibility in the normal genome that was previously thought impossible. The possibility, however, that the generation of tumor heterogeneity is a reflection of 'normal' processes should be remembered when considering methods to limit it.

Stability of tumor cell subpopulations

In view of the above discussion on the origins of tumor cell subpopulations, it may seem that a consideration of subpopulation stability is unnecessary. Many investigators, however, seem to feel that the processes generating diversity somehow cease at the moment they have obtained a cell subpopulation. This expectation is the source of experimental frustration, scientific conflict, and intellectual error. For example, the continuing capacity of tumor cells to generate diversity is not appreciated by investigators who think that cells from metastatic foci should be more metastatic or less heterogeneous than their parent tumors (121). Although this can be so, particularly for parent tumors which are not already metastasizing at a very high rate (122), cells in individual metastases may also be heterogeneous for all the reasons discussed above and for some to be described below. Tumor heterogeneity is a dynamic process!

Suffice it to say that our experience (47), as well as that of others (123–125), shows that individual subpopulations and clones thereof are heterogeneous in their stability. Furthermore, normal cell clones can be similarly unstable (96). Tumor subpopulation changes can be sudden (47, 124) or gradual (47) and to a more (47, 123–125) or less (47,

124) malignant phenotype. Changes can occur after in vitro (47, 123, 124) or in vivo (113, 125) passage. The degree of stability seems to vary with the phenotype; in our experience, at least, ability to metastasize is one of the most labile characteristics. Whether this is due to the multifactorial requirements of the metastatic process, where any of many changes could affect the outcome, or whether it reflects differences in the mechanisms responsible for generation of variation in different phenotypes, requires further study. A most interesting finding is that of Poste et al. (126) who showed that instability in the metastatic phenotype of B16 melanoma cells became evident after cloning, whereas individual clones growing together as mixed cell populations retained their characteristic degree of metastatic ability. This observation is one of a series showing that tumor cell interactions can influence subpopulation behavior.

Tumor cell subpopulation interactions

Demonstration of tumor cell heterogeneity focuses on differences among multiple cell subpopulations. These differences are shown most convincingly when the subpopulations are grown and compared in isolation from each other. Tumor cells and cell subpopulations do not, however, exist independently of each other, but rather as parts of mixed cell populations. Cancer development and growth are 'group phenomena'. Cellular interactions can affect the frequency of initiation, as well as growth into overt cancers (127). Not only can normal and tumor cells influence each other's growth (128–134), there are well-described interactions between embryonic cells (127) and between normal adult cells (135). Thus, the existence of interactions between tumor cell subpopulations is not unexpected.

Early investigators of tumor heterogeneity, most prominently Hauschka (136), commented that the growth of isolated sublines of a tumor was sometimes faster than the growth of the parent tumor from which they came and suggested the existence of mechanisms within the parent tumor to control growth of the more vigorous subpopulations. Similar observations have been made by more recent workers (21, 137). Makino showed that two different sarcomas could control each other's growth (138), and Cheshire noted that the growth rate of single, spontaneously arising C_3H mouse mammary tumors was generally faster than were the rates of the first tumors appearing on mice which had developed multiple tumors (139).

Our laboratory has described a number of subpopulation interactions that influence growth. Using a protocol in which cells of two different subpopulations from a mouse mammary tumor are injected on opposite sides of a single mouse, we have shown that, depending upon the particular subpopulations injected, one subpopulation can either enhance or retard growth of another (140, 141). The mechanism of interaction of one such combination is apparently host-mediated in that it is abrogated by immunosuppression (141). The basis for this interaction is the ability of one subpopulation to induce immunity to itself and to the other subpopulation, whereas the second subpopulation is nonimmunogenic. We have found other types of growth interactions between mammary tumor subpopulations as well. These interactions can be detected in vitro and so do not depend upon host factors. Cocultures of tumor subpopulations that by themselves have different growth rates grow at the rate of either one or the other subpopulation. Thus, under some conditions one subpopulation stimulates growth of another; under other conditions the second subpopulation inhibits growth of the first (140, 142). Furthermore, conditioned media from certain subpopulations are inhibitory to growth of other, but not all, subpopulations from the same tumor (140, 142). Such media are inhibitory to cells of other mammary tumors, including a human breast cancer line, but are not inhibitory to a number of cell lines of nonmammary origin.

Our data indicate many mechanisms by which tumor subpopulations can affect each other's growth (142). Some of these mechanisms are undoubtedly analogous to those used by normal cells during embryonic development and organogenesis (135). Our conditioned media factor shares certain characteristics with chalones. Furthermore, both normal and cancer cells produce a wealth of factors

that can influence growth of other cells, as well as of themselves (135, 143–146). Insofar as the production of these factors may be distributed heterogeneously among tumor cell subpopulations, they may be able to effect subpopulation interactions.

Growth characteristics are not the only ones influenced by tumor subpopulation interactions. The ability of one subpopulation to alter drug sensitivity of another subpopulation will be described below. Interactions affecting immune reactivity have also been reported. Mixtures of immunogenic and nonimmunogenic tumor cells can either induce or not induce overall immunity to tumor challenge, depending upon the relative proportions of the two types of cells (147). The immunogenicity of AKR leukemia cells is enhanced by immunization with mixtures of subpopulations that express different antigens (20). Alternatively, one could imagine a situation in which 'antigenic competition' between tumor cell subpopulations might reduce the immunogenicity of any one of them. We have found that mixtures of tumor cell subpopulations can induce patterns of cross-reactive immunity not predictable on the basis of the immune responses induced by either subpopulation alone (148). The basis for these types of interactions have been discussed by Miller recently in this series (45).

An important class of tumor cell interactions are those which affect malignant behavior. Browning reported that development in C_3H mice of multiple mammary tumors interfered with progression of the first tumor to 'autonomy', defined as the ability to grow across histocompatibility and species barriers (149). Klein and Klein (150) demonstrated that solid tumor variants capable of independently growing in ascites fluid could be masked by non-ascitic variants, so that the mixture did not grow as an ascites. The basis of this interaction was nutritional.

There are numerous observations on the effect of a primary tumor on the development of metastases (151–156). Although the mechanism of these interactions is often assumed to be immunological, this is by no means certain (157). Surely, the various growth interaction factors discussed above may play a role. Furthermore, tumor subpopulation interactions may actually affect expression of the metastatic phenotype, not just the growth of established metastases. Mention has already been made of the observation that the clonal instability of the metastatic phenotype is somehow stabilized in mixtures of clones (126). Fred Miller in our laboratory has also shown that mouse mammary tumor cell subpopulations which do not metastasize when growing alone in mice will do so in the presence of metastatic subpopulations (158). This is so even when the 'nonmetastatic' cells are implanted subcutaneously and the 'metastatic' cells are injected intraveneously. Nodules of metastatic growth, containing clonogenic cells of both types, can be found in the lung. Some metastases are mixtures of the two cell types, whereas in some nodules only one or the other type can be found. In addition to demonstrating that metastases need not arise from single cells (159), Miller's experiments illustrate another reason why cells isolated from metastases may not be more metastatic than cells from the primary tumor (see above).

The mechanisms whereby a metastatic subpopulation could induce metastasis by 'nonmetastatic' cells are as yet speculative: Nonmetastatic cells may be carried along as 'innocent bystanders' in clumps of metastatic cells. Perhaps a central effect on host immunity is involved. Metastatic cells could secrete or shed substances that affect any of the many steps in the metastatic cascade. Growth of tumors at two different sites (perhaps reflecting organ site preferences of two different subpopulations) has been shown to influence collagenolytic activity in tumor extracts (160). The ability of vesicles prepared from the metastatic F10 line of B16 melanoma to induce metastatic behavior in F1 cells has been reported (161). Vesicles shed from some tumors have been shown to carry procoagulent activity which could perhaps cause a fibrin gel to be formed around otherwise nonmetastatic cells, thereby protecting them from host defense (162). Furthermore, fibrin may induce angiogenesis (162), an essential step in metastasis.

It is clear that there are many ways in which tumor subpopulations can influence each other's growth and behavior. Both host and tumor factors are involved. Some interactions require cell contact,

others act systemically (142). Which mechanisms are responsible in any given circumstance depend upon the characteristics of the particular subpopulations involved and of the environment in which they are interacting. Tumor subpopulations are opportunistic; they interact by whatever means are at hand. As with any society, the ways in which the various parts influence each other can be subtle, but also have profound consequences.

Progression and tumor heterogeneity

Previous mention was made of the concept of 'tumor progression' as it related to the heterogeneity seen in the same tumor as a function of time. Progression was defined by Foulds (1) as stepwise neoplastic development through *qualitatively* different stages. The term 'progression' is confusing. It does not necessarily mean an increase in malignant behavior; tumors can 'regress'. It also does not refer to continuous growth in space or in time. Foulds formulated six 'rules' of progression: (a) progression is independent in multiple tumors of the same host, (b) progression is independent in different characteristics of the same tumor, (c) progression is independent of tumor growth, (d) progression can occur by gradual or by abrupt steps, (e) progression can occur by alternate paths, and (f) progression does not always reach an end point at the death of the host.

Fould's definition and rules of progression were based on extensive experimental and clinical documentation of how cancers in fact behave. They are descriptive and do not suggest a mechanism. Taken at their extremes, there are two basic mechanisms which have been used to explain the phenomenon – progression occurs because new characteristics are acquired during tumor growth or it occurs because subpopulations of tumor cells with different characteristics, which existed very early in the life of the tumor, are *selected* by changing environmental conditions (163).

The concepts of tumor progression have been studied classically in regard to two characteristics: the loss of hormone dependence in initially dependent tumors and the acquisition of metastatic capability. In both cases there is extensive evidence that subpopulations of either hormone independent or metastatic tumor cells exist prior to progression.

The presence of hormone independent cells within early, dependent tumors has been shown in estrogen-dependent mouse, rat, and human breast cancers. In human tumors this has been shown by a heterogeneous distribution of hormone receptors (68–70); in animal tumors this has been demonstrated in addition by the ability to grow in castrated hosts (33, 35, 38, 164, 165). Loss of hormone dependence may be accompanied by a shift in the relative proportion of tumor cells with different morphology (165). An elegant recent study by Michalides and associates (35) also demonstrated the presence of autonomous tumor cells in hormone-dependent strain GR mouse tumors by using MuMTV DNA probes to detect minor subpopulations.

Evidence for the coexistence of hormone-dependent and independent subpopulations has been presented for prostatic cancer. Human prostatic cancer undergoes a morphological shift during transition to hormone independence (166). Isaacs and Coffey (167) used fluctuation analysis to demonstrate formally the presence of androgen-independent cells within the androgen-dependent Dunning R-3327-H rat prostatic adenocarcinoma line. This study also showed that the independent cells were not distributed evenly within the tumors but were more prevalent in some areas than in others.

That metastatic capacity is not distributed randomly among all cells of a tumor but instead is a property of only some tumor subpopulations has been thoroughly demonstrated by the work of Fidler and associates (18, 19, 23, 25, 26, 168). These studies have utilized tumors of recent origin, as well as long-term lines. Wang and co-workers (56) used marker chromosomes to demonstrate the existence of metastatic subpopulations within a methylcholanthrene-induced tumor which had been passaged only twice. Chromosomal analysis has also been used to demonstrate selection of subpopulations with different growth site preferences within a rat leukemic line (54).

Although the presence of multiple subpopula-

tions within primary cancers is in keeping with the hypothesis that progression is the consequence of variant selection, it does not prove it. That such a mechanism can operate, however, was shown by Sluysers and co-workers (169) who injected mixtures of hormone-dependent and hormone-sensitive mammary tumor cells into normal and castrated hosts. Even when only 10% of the tumor cells were hormone independent, the growth of the tumors in both kinds of hosts was controlled by the autonomous cells. Other workers have demonstrated chromosomal changes accompanying tumor progression which also suggest that selection of variant subpopulations is the underlying mechanism (170). Thus, it seems likely that progression reflects the selection of subpopulations, which are present early in tumorigenesis and also may arise at any point during tumor growth and development. This mechanism would seem to provide the simplest explanation for Fould's 'rules' (1, 171). The fact that any rules can be defined, however, indicates that even within those twin phenomena of variability – heterogeneity and progression – there is an overlying homogeneity which provides a 'controlled' process. Kiang and associates (172) have described a cyclical variability in certain tumor cell characteristics during progression of hormone-dependent GR mouse mammary tumors to hormone independency. Vaage (173) has shown in repeated serial transplantation experiments that individual C_3H mammary tumors undergo 'progression' in a highly reproducible way; certain characteristics appear in the same generations, as if on schedule. These observations suggest that 'genetic variability and selection' is not the whole story in progression. Tumor progression is not a chaotic free-for-all. Indeed, Poste et al. (126) have shown that genetic variability is subject to regulatory influences which apparently involve tumor subpopulation interactions. Kiang et al. (172) proposed that their cyclic phenomena argue for the 'continued presence of regulatory mechanisms among various cell subpopulations'. Thus, just as for other characteristics discussed above, the behavior of neoplastic cell populations is that of an interactive ecosystem in which the characteristics of the whole is determined, but not fully described, by the characteristics of the parts.

Therapeutic implications of tumor heterogeneity

The observation that tumors are made up of subpopulations of varying characteristics poses a problem for the development of effective therapeutics. It has been demonstrated that tumor subpopulations differ in susceptibility to chemotherapy (15, 46, 73–75, 81, 174–183), radiation therapy (184–187), and immunotherapy (14, 20, 40–43, 64–66, 76, 77, 188–191). Furthermore, heterogeneity in the metastatic phenotype results in differential sensitivity between primary tumors and metastases. It may be that a metastatic tumor is composed of tumor subpopulations which make up only a small proportion of the primary tumor. Subpopulations which were never present in the primary tumor may exist in metastases, a result of the generation of new variants by cells in the metastatic nodule. In addition, differential sensitivity to drugs (73, 179, 183) and differential antigen expression (77, 189) have been shown between different metastases. It is not surprising that the metastases may bear little resemblance to the primary either in chemosensitivity to specific drugs or in antigen expression. Thus, specific immunotherapy with vaccines prepared from the autologous primary tumor may be doomed to failure (41). For the same reason, it may be difficult to individualize chemotherapy based on in vitro tests to determine the drug sensitivity of the primary tumor.

The problem of selecting effective therapy for heterogeneous tumors may be further compounded by the existence of interactions between tumor subpopulations, as well as between tumor cells and normal cells, which affect the measured sensitivity of the whole tumor. We have shown that subpopulations of a mouse mammary tumor which differ in sensitivity to chemotherapeutic agents can interact in such a way that the apparent sensitivity of one subpopulation is changed in the presence of the other (192). Interactions between cells may act through metabolic processes affecting drug metabolism, through factors affecting cell growth or through immune mechanisms. For example, the well known phenomenon of 'metabolic cooperation', a process by which small molecules pass between cells in contact, presumably through gap

junctions (193, 194), is one way in which interactions between cells can affect response to therapy. Metabolic cooperation could result in the rescue of a sensitive cell by molecules from a resistant cell, or in the death of a resistant cell due to passage of molecules from a sensitive cell.

Tumor heterogeneity can influence the predictive value of laboratory tests for drug sensitivity. To date, the most widely used in vitro test for predicting the drug sensitivity of a tumor is the clonal assay of Salmon et al. (195). A virtue of this assay, in which cells are allowed to form colonies in soft agar after treatment which various chemotherapeutic agents, is that it measures the growth of tumor cells; very few normal cells will form colonies in soft agar. However, one major disadvantage of the assay, from our point of view, is that relatively few tumor cells form colonies either. The assay is thus highly selective for a minor subpopulation(s) of cells. It is not apparent that the minority subpopulations whose chemosensitivity is being measured are always more invasive or metastatic and thus more important to eliminate by therapy. An additional problem in a clonal assay is disruption of subpopulation interactions which may be important in the effect of the chemotherapy in situ.

Another widely used test to predict drug sensitivity of human tumors involves the growth of tumors as xenografts in 'nude' or immunosuppressed mice (196). This assay has some advantages in that it allows interactions between tumor cells to take place, and it allows pharmacokinetics of drug delivery and drug metabolism to be somewhat more like that in the patient, but it is also highly selective for subpopulations able to grow as xenografts.

We are attempting to devise an in vitro assay for drug sensitivity which acknowledges the principles of heterogeneity (197). We are testing the ability of drugs to inhibit the 3-dimensional growth of tumor cells in collagen gels. Tumor cells may either be suspended and embedded as a bolus, or embedded as a small piece (< 1 mm^3) without dissociation. The advantages of this assay are that tumor cell interactions and three-dimensional architecture can be maintained, and that growth on collagen is less selective than cloning in soft agar, especially for hard-to-clone tumors such as breast cancer. In addition, when tumor pieces are used, sampling effects can be controlled directly by examining the variation in drug sensitivity and growth in pieces which were close together or far apart in the original tumor.

Tumor heterogeneity in drug sensitivity, and the presence of multiple subpopulations within single neoplasms, need consideration in development of treatment strategies as well as in sensitivity testing. In itself tumor heterogeneity provides a rationale for combination chemotherapy and combination modality therapy, since subpopulations resistant to one treatment might be sensitive to another. Goldie and Coldman have developed a mathematical model of drug resistance in a tumor based on the hypothesis that phenotypic changes in drug sensitivity are the result of drug resistant cells arising with constant frequency (198). The event resulting in a resistant cell is presumed to be a random one, occuring with a certain probability based on the Luria and Delbruck model (199). The time at which a resistant cell arises will be a function of the growth curve and rate of variant production. As a result of their analysis, these authors show that sometime in the growth of a tumor, the tumor population will go from a high probability to a low probability of having no resistant clones. The higher the variation rate, the earlier in the growth of the tumor will this transition occur. The implication of this analysis is that adjuvant chemotherapy should be started as early as possible after detection of malignancy, perhaps even before surgery. One can also see that possibly effective drugs should not be 'held back' for use after failure of first-line chemotherapy; rather, as many drugs as may be effective should be used as early as possible. Since variant production can occur at any time, it may be also that new variants will arise that are sensitive to drugs which had been previously ineffective.

Now that combination chemotherapy is standard practice for many tumors, great interest has developed in searching for therapeutic synergism in drug combinations and in avoiding antagonistic combinations. These interactions between drugs have been shown to be affected by timing and order of administration (200, 201). Much of the research directed toward explaining the mechanism by which

drug combinations interact has been based on effects on homogeneous cell populations; thus far, postulated interactions between drugs have been suggested as due to metabolic interactions within a single cell (202–206). We suggest that some observed synergisms between drugs seen in animal or clinical trials may be due to the drugs' effects on different cell populations or to changing interactions between tumor cells or between tumor and normal cells.

The above considerations suggest that although tumor heterogeneity poses a problem to cancer therapy, it also may prove possible to learn to use it in a strategic way. Tumor subpopulation interactions may provide the basis for an in situ 'biological response modification' of tumor growth. Growth factors produced by one subpopulation may, by affecting growth of other populations, alter response to chemotherapy. An immunogenic subpopulation can induce immunity to otherwise non-immunogenic subpopulations thereby extending the range of specific immunotherapy. It may also be possible to influence the extent of tumor heterogeneity and thus affect therapeutic response. Leith and colleagues have shown how the use of agents which effect differentiation of tumor cells may result in the production of more homogeneous tumor cell populations which respond uniformly to drugs or irradiation (185). Thus, knowledge of how cancer cell populations develop and interact can provide clues to new approaches to therapy. The 'problem' of tumor heterogeneity may come to be seen as the 'solution' to control of neoplastic growth.

Acknowledgment

This research was supported by USPHS Grant CA-27419 and the E. Walter Albachten bequest.

References

1. Foulds L: Neoplastic development, 2 vols. Academic Press, New York, 1969, 1975.
2. Vaupel PW, Frinak S, Bicher HI: Heterogeneous oxygen partial pressure and pH distribution in C_3H mouse mammary adenocarcinoma. Cancer Res (41): 2008–2013, 1981.
3. Dethlefsen L: The growth dynamics of murine mammary tumor cells in situ. *In:* McGrath CM, Brennan MJ, Rich MA (eds) Cell biology of breast cancer. New York, Academic Press 1980, pp 145–160.
4. Bosman HB, Winston RA: Synthesis of glycoprotein, glycolipid, protein, and lipid in synchronized L5178Y cells. J Cell Biol (45): 23–33, 1970.
5. Pasternak CA, Warmsley AMH, Thomas DB: Structured alterations in the surface membrane during the cell cycle. J Cell Biol (50): 562–564, 1971.
6. Cikes M, Klein G: Quantitative studies of antigen expression in cultured murine lymphoma cells. I. Cell-surface antigens in 'Asynchronous' cultures. J Natl Cancer Inst (49): 1599–1606, 1972.
7. Panem S, Schauf V: Cell-cycle dependent appearance of murine leukemia – sarcoma virus antigens. J Virol (13): 1169–1175, 1974.
8. Everson LK, Plocinik BA, Rogentine GN: HL-A expression on the G_1, S, and G_2 cell-cycle stages of human lymphoid cells. J Natl Cancer Inst (53): 913–920, 1974.
9. Shipley WU: Immune cytolysis in relation to the growth cycle of chinese hamster cells. Cancer Res (31): 925–929, 1971.
10. Lerner RA, Oldstone MBA, Cooper NR: Cell cycle-dependent immune lysis of Moloney virus-transformed lymphocytes: presence of viral antigen, accessibility to antibody, and complement activation. Proc Nat Acad Sci USA (68): 2584–2588, 1971.
11. Valeriote F, van Putten L: Proliferation dependent cytotoxicity of anticancer agents: a review. Cancer Res (35): 2619–2630, 1975.
12. Suzuki N, Withers HR, Koehler MW: Heterogeneity and variability of artificial lung colony forming ability among clones from mouse fibrosarcoma. Cancer Res (38): 3349–3351, 1978.
13. Weiss L: Cancer cells in primary tumors and their metastases. *In:* McGrath CM, Brennan MJ, Rich MA (eds) Cell biology of breast cancer. New York, Academic Press, 1980, pp 189–205.
14. Prehn RT: Analysis of antigenic heterogeneity within individual 3-methylcholanthrene-induced mouse sarcomas. J Natl Cancer Inst (45): 1039–1045, 1970.
15. Hakansson L, Tropé C: On the presence within tumors of clones that differ in sensitivity to cytostatic drugs. Acta Pathol Microbiol Scand Suppl Section A (82): 35–40, 1974.
16. Fidler IJ, Hart IR: Biological and experimental consequences of the zonal composition of solid tumors. Cancer Res (41): 3266–3267, 1981.
17. Gray JM, Pierce GB: Relationship between growth rate and differentiation of melanoma in vivo. J Natl Cancer Inst (32): 1201–1211, 1964.
18. Fidler IJ, Kripke ML: Metastasis results from preexisting

variant cells within a malignant tumor. Science (197): 893–895, 1977.

19. Fidler IJ, Gruys E, Cifone MA, Barnes Z, Bucana C: Demonstration of multiple phenotypic diversity in a murine melanoma of recent origin. J Natl Cancer Inst (67): 947–956, 1981.
20. Olsson L, Ebbesen P: Natural polyclonality of spontaneous AKR leukemia and its consequences for so-called specific immunotherapy. J Natl Cancer Inst (62): 623–627, 1979.
21. Mathieson BJ, Zatz MM, Sharrow SO, Asofsky R, Logan W, Kanellopoulos-Langevin C: Separation and characterization of two component tumor lines within the AKR lymphoma, AKTB-1, by fluorescence-activated cell sorting and flow microfluorometry analysis. J Immunol (128): 1832–1838, 1982.
22. Mitelman F: The chromosomes of fifty primary Rous rat sarcomas. Heriditas (69): 155–186, 1971.
23. Nicolson GL, Brunson KW, Fidler IJ: Specificity of arrest, suvival, and growth of selected metastatic variant cell lines. Cancer Res (38): 4105–4111, 1978.
24. Varani J, Orr W, Ward PA: A comparison of the migration patterns of normal and malignant cells in two assay systems. Am J Pathol (90): 159–172, 1978.
25. Kripke, ML, Gruys E, Fidler IJ: Metastatic heterogeneity of cells from an ultraviolet light-induced murine fibrosarcoma of recent origin. Cancer Res (38): 2962–2967, 1978.
26. Raz A, Hanna N, Fidler IJ: In vivo isolation of a metastatic tumor cell variant involving selective and non-adaptive processes. J Natl Cancer Inst (66): 183–189, 1981.
27. Henderson JS, Rous P: The plating of tumor components on the subcutaneous expanses of young mice. J Exp Med (115): 1211–1229, 1962.
28. Dominguez OV, Huseby RA: Heterogeneity of induced testicular interstitial cell tumors of mice as evidenced by steroid biosynthetic enzyme activities. Cancer Res (28): 348–353, 1968.
29. Pierce GB: Cellular heterogeneity of cancers. *In*: T'so POP, DiPaolo JA (eds) World symposium on model studies in chemical carcinogenesis. New York, Dekker, 1974, pp 463–472.
30. Dexter DL, Kowalski HM, Blazar BA, Fligiel Z, Vogel R, Heppner GH: Heterogeneity of tumor cells from a single mouse mammary tumor. Cancer Res (38): 3174–3181, 1978.
31. Danielson KG, Anderson LW, Hosick HL: Selection and characterization in culture of mammary tumor cells with distinctive growth properties in vivo. Cancer Res (40): 1812–1819, 1980.
32. Tseng MT: Ultrastructure of the hormone-dependent N-Nitrosomethylurea-induced mammary carcinoma of the rat. Cancer Res (40): 3112–3115, 1980.
33. Macinnes JI, Chan ECM, Percy DH, Morris VL: Mammary tumors from GR mice contain more than one population of mouse mammary tumor virus-infected cells. Virol (113): 119–129, 1981.
34. Colcher D, Hand PH, Teramoto YA, Wunderlich D, Schlom J: Use of monoclonal antibodies to define the diversity of mammary tumor viral gene products in virions and mammary tumors of the genus *Mus*. Cancer Res (41): 1451–1459, 1981.
35. Michalides R, Wagenaar E, Sluyser M: Mammary tumor virus DNA as a marker for genotypic variance within hormone-responsive GR mouse mammary tumors. Cancer Res (42): 1154–1158, 1982.
36. Kobori O, Oota K: Neuroendocrine cells in serially passaged rat stomach cancers induced by MNNG. Int J Cancer (23): 536–541, 1979.
37. Hager J, Fligiel S, Stanley W, Richardson AM, Heppner GH: Characterization of a variant producing tumor cell line from a heterogeneous strain BALB/cfC_3H mouse mammary tumor. Cancer Res (41): 1293–1300, 1981.
38. Sluyser M, Evers SG, DeGoeij CCJ: Sex hormone receptors in mammary tumors of GR mice. Nature (263): 386–389, 1976.
39. Pimm MV, Baldwin RW: Antigenic differences between primary methylcholanthrene-induced rat sarcomas and post-surgical recurrences. Int J Cancer (20): 37–43, 1977.
40. Fuji H, Mihich E, Pressman D: Differential tumor immunogenicity of L1210 and its sublines. J Immunol (119): 983–986, 1977.
41. Killion JJ: Immunotherapy with tumor cell subpopulations. Cancer Immunol Immunother (4): 115–119, 1978.
42. Schirrmacher V, Bosslet K, Shantz G, Claver K, Hubsch D: Tumor metastases and cell-mediated immunity in a model system in DBA/2 mice. IV. Antigenic differences between a metastasizing variant and the parental tumor line revealed by cytotoxic T lymphocytes. Int J Cancer (23): 245–252, 1979.
43. Miller FR, Heppner GH: Immunologic heterogeneity of tumor cell subpopulations from a single mouse mammary tumor. J Natl Cancer Inst (63): 1457–1464, 1979.
44. Miller BE, Miller FR, Leith J, Heppner GH: Growth interaction in vivo between tumor subpopulations derived from a single mouse mammary tumor. Cancer Res (40): 3977–3981, 1980.
45. Miller FR: Intratumor immunologic heterogeneity. Cancer Metastasis Rev (1): 319–334, 1982.
46. Heppner GH, Dexter DL, DeNucci T, Miller FR, Calabresi P: Heterogeneity in drug sensitivity among tumor cell subpopulations of a single mammary tumor. Cancer Res (38): 3758–3763, 1978.
47. Miller FR, Miller BE, Heppner GH: Metastatic heterogeneity of a single mouse mammary tumor: frequency, stability, and site dependency. Invasion Metastasis (in press).
48. Varani J, Orr W, Ward PA: Adhesive characteristics of tumor cell variants of high and low tumorigenic potential J Natl Cancer Inst (64): 1173–1178, 1980.
49. Soule HD, Maloney T, McGrath CM: Phenotypic variance among cells isolated from spontaneous mouse mam-

mary tumors in primary suspension culture. Cancer Res (41): 1154–1164, 1981.
50. Rios A, Laux D, Heppner GH: Patterns of lymphocyte infiltration in tumor sublines of a single mammary adenocarcinoma. Proc AACR (23): 1021 (abstract), 1982.
51. Levan A, Hauschka TS: Endomitotic reduplication mechanisms in ascites tumors of the mouse. J Natl Cancer Inst (14): 1–21, 1953.
52. Makino S: Further evidence favoring the concept of the stem cell in ascites tumors of rats. Ann N Y Acad Sci (63): 818–830, 1956.
53. Becker FF, Klein KM, Wolman SR, Asofsky R, Sell S: Characterization of primary hepatocellular carcinomas and initial transplant generations. Cancer Res (33): 3330–3338, 1973.
54. Ishidate M, Aoshima M, Sakurai Y: Population changes of a rat leukemia by different routes of transplantation. J Natl Cancer Inst (53): 773–781, 1974.
55. Nowell PC: The clonal evolution of tumor cell populations. Science (194): 23–28, 1976.
56. Wang N, Yu SH, Liener IE, Hebbel RP, Eaton JW, McKhann CF: Characterization of high and low metastatic clones derived from a methylcholanthrene-induced murine fibrosarcoma. Cancer Res (42): 1046–1051, 1982.
57. Hager JC, Heppner GH: Heterogeneity of expression and induction of mouse mammary tumor virus antigens in mouse mammary tumors. Cancer Res (42): 4325–4329, 1982.
58. Geier GR, Schwarz JA, Schlag P: Cytologic uniformity of breast cancer from different locations: a pattern analyses study. Expl Cell Biol (47): 241–249, 1979.
59. Parbhoo SP: Heterogeneity in human mammary cancer. *In*: Stoll BA (ed) Systemic control of breast cancer. London, William Heinemann Medical Books, 1981, pp 55–77.
60. Ewing SL, Sumner HW, Ophoven JJ, Mayer JE, Humphrey EW: Small cell anaplastic carcinoma with differentiation: a report of 14 cases (abstract). Lab Invest (42): 115, 1980.
61. McDowell EM, Sorokin SP, Hoyt RF, Trump BF: An unusual bronchial carcinoid tumor: light and electron microscopy. Human Pathol (12): 338–348, 1981.
62. Stich HF, Florian SF, Emson HE: The DNA content of tumor cells. I. Polyps and adenocarcinoma of the large intestine of man. J Natl Cancer Inst (24): 471–482, 1960.
63. Vindelv LL, Hansen HH, Christensen HJ, Spang-Thomsen M, Hirsch FR, Hansen M, Nissen NI: Clonal heterogeneity of small-cell anaplastic carcinoma of the lung demonstrated by flow-cytometric DNA analysis. Cancer Res (40): 4295–4300, 1980.
64. Byers VS, Johnston JO: Antigenic differences among osteogenic sarcoma tumor cells taken from different locations in human tumors. Cancer Res (37): 3173–3183, 1977.
65. Tan MN, Shimano T, Chu TM: Differential localization of human pancreas cancer-associated antigen and carcinoembryonic antigen in homologous pancreatic tumoral xenograft. J Natl Cancer Inst (67): 563–569, 1981.
66. Horan Hand P, Nuti M, Colcher D, Schlom J: Monoclonal antibodies to tumor associated antigens define antigenic heterogeneity among human mammary cancer cell populations (submitted for publication).
67. Weiss MA, Michael JG, Pesce AJ, DiPersio L: Heterogeneity of B_2-microglobulin in human breast carcinoma. Lab Invest (45): 46–57, 1981.
68. Lee SH: Cytochemical study of estrogen receptor in human mammary cancer. Am J Clin Pathol (70): 197–203, 1978.
69. Pertschuk LP, Tobin EH, Brigati DJ, Kim DS, Bloom ND, Gaetjens E, Berman PJ, Carter AC, Degenstein GA: Immunofluorescent detection of estrogen receptors in breast cancer. Cancer (41): 907–911, 1978.
70. Brennan MJ, Donegan WL, Appleby DE: The variability of estrogen receptors in metastatic breast cancer. Am J Surg (137): 260–262, 1979.
71. Lippman SM, Mendelsohn G, Trump DL, Wells SA, Baylin SB: The prognostic and biological significance of cellular heterogeneity in medullary thyroid carcinoma: a study of calcitonin, L-Dopa decarboxylase, and histaminase. J Clin Endo Met (54): 233–240, 1982.
72. Baylin SB, Weisburger WR, Eggleston JC, Mendelsohn G, Beaven MA, Abeloff MD, Ettinger DS: Variable content of histaminase, L-Dopa decarboxylase and calcitonin in small-cell carcinoma of the lung. New Engl J Med (299): 105–110, 1978.
73. Sirackỳ J: An approach to the problem of heterogeneity of human tumor-cell populations. Br J Cancer (39): 570–577, 1979.
74. Barranco SC, Ho DHW, Drewinko B, Romsdahl MM, Humphrey RM: Differential sensitivity of human melanoma cells grown in vitro to arabinosylcytosine. Cancer Res (32): 2733–2736, 1972.
75. Barranco SC, Drewinko B, Humphrey RM: Differential response by human melanoma cells to 1,3-bis-(2-chloroethyl)-1-nitrosourea and bleomycin. Mutation Res (19): 277–280, 1973.
76. Sorg C, Bruggen J, Seibert E, Macher E: Membrane-associated antigens of human malignant melanoma IV: changes in expression of antigens on cultured melanoma cells. Cancer Immunol Immunother (3): 259–271, 1978.
77. Albino AP, Lloyd KO, Houghton AN, Oettgen H, Old LJ: Heterogeneity in surface antigen and glycoprotein expression of cell lines derived from different melanoma metastases of the same patient. J Exp Med (154): 1764–1778, 1981.
78. Aubert C, Rougé F, Galindo JR: Tumorigenicity of human malignant melanocytes in nude mice in relation to their differentiation in vitro. J Natl Cancer Inst (64): 1029–1040, 1980.
79. Brattain MG, Fine WD, Khaled FM, Thompson J, Brattain DE: Heterogeneity of malignant cells from a human colonic carcinoma. Cancer Res (41): 1751–1756, 1981.
80. Dexter DL, Spremulli EN, Fligiel Z, Barbosa JA, Vogel R,

VanVoorhees A, Calabresi P: Heterogeneity of cancer cells from a single human colon carcinoma. Am J Med (71): 949–956, 1981.

81. Chu MY: Tumor cell heterogeneity in human lung carcinoma. Proc AACR (20): 151 (abstract), 1979.
82. Mackintosh FA, Louie AC, Evans TL, Amylon MD, Sikic BI: Clonal heterogeneity in a human ovarian adenocarcinoma. Proc AACR (22): 184 (abstract), 1981.
83. Kajiji SM, Meitner PA, Bogaars HA, Dexter DL, Cummings FJ, Calabresi P, Turner MD: Establishment of a fast growing variant of human pancreatic cancer (HPC). Proc AACR (23): 119 (abstract), 1982.
84. Shapiro JR, Yung WA, Shapiro WR: Isolation, karyotype, and clonal growth of heterogeneous subpopulations of human malignant gliomas. Cancer Res (41): 2349–2359, 1981.
85. Wikstrand CJ, Bigner SP, Bigner DD: Antigenic heterogeneity of an established human glioma cell line (HGCL) and eight single cell derived clones as defined by specific anti-glioma monoclonal antibodies (MCA). Proc AACR (23): 1070 (abstract), 1982.
86. Kimball PM, Brattain MG: Isolation of a cellular subpopulation from a human colonic carcinoma cell line. Cancer Res (40): 1574–1579, 1980.
87. Okamura S, Chechik BE, Lee C, Gelfand EW, Mak TW: Heterogeneity of human thymocytes and a malignant T-lymphoblast cell line, MOLT-3. Cancer Res (41): 1664–1668, 1981.
88. Butler WB, Berlinski PJ, Kelsey WH, Toehniges MM: Heterogeneity of the human breast cancer cell line MCF-7. Proc AACR (23): 931 (abstract), 1982.
89. Fialkow PJ: Clonal origin of human tumors. Ann Rev Med (30): 135–143, 1979.
90. Friedman JM, Fialkow PJ: Viral 'tumorigenesis' in man: cell markers in condylomata acuminata. Int J Cancer (17): 57–61, 1976.
91. Tanooka H, Tanaka K: Evidence for single-cell origin of 3-methylcholanthrene-induced fibrosarcomas in mice with cellular mosaicism. Cancer Res (42): 1856–1858, 1982.
92. Reddy AL, Fialkow PJ: Multicellular origin of fibrosarcomas in mice induced by the chemical carcinogen 3-methylcholanthrene. J Exp Med (150): 878–887, 1979.
93. Till JE, McColloch EA: Hemopoietic stem cell differentiation. Biochim Biophys Acta (605): 431–459, 1980.
94. Good RA: Structure – function relations in the lymphoid system. *In*: Bach FH, Good RA (eds) Clinical immunobiology, vol 1, New York, Academic Press, 1972, pp 1–28.
95. St. George JA, Cardiff RD, Young LJT, Faulkin LJ: Immunocytochemical distribution of mouse mammary tumor virus antigens in BALB/cfC$_3$H mammary epithelium. J Natl Cancer Inst (63): 813–820, 1979.
96. Griffen JE, Allman DR, Durrant JL, Wilson JD: Variation in steroid 5α-reductase activity in cloned human skin fibroblasts. J Bio Chem (256): 3662–3666, 1981.
97. Ogawa K, Solt DB, Farber E: Phenotype diversity as an early property of putative preneoplastic hepatocyte populations in liver carcinogenesis. Cancer Res (40): 725–733, 1980.
98. Ashley RL, Cardiff RD, Mitchel DJ, Faulkin LJ, Lund JK: Development and characterization of mouse hyperplastic mammary outgrowth lines from BALB/cfC$_3$H hyperplastic alveolar nodules. Cancer Res (40): 4232–4242, 1980.
99. Boone CW, Vembu D, White BJ, Takeichi N, Paranjpe M: Karyotypic, antigenic, and kidney-invasive properties of cell lines from fibrosarcomas arising in C$_3$H/10T½ cells implanted subcutaneously attached to plastic plates. Cancer Res (39): 2172–2178, 1979.
100. Kakunaga T, Crow JD: Cell variants showing differential susceptibility to ultraviolet light-induced transformation. Science (209): 505–507, 1980.
101. Cifone M, Fidler IJ: Increasing metastatic potential is associated with increasing genetic instability of clones isolated from murine neoplasms. Proc Natl Acad Sci USA (78): 6249–6252, 1981.
102. Jansson B, Révész L: A deductive approach to the analysis of the growth of ascites tumor cell populations. *In*: Busch H (ed) Methods in cancer research, Vol XIII. New York, Academic Press, 1976, pp 227–290.
103. Smith BL, Sager R: Multistep origin of tumor-forming ability in chinese hamster embryo fibroblast cells. Cancer Res (42): 389–396, 1982.
104. Canaani E, Aaronson SA: Restriction enzyme analysis of mouse cellular type C viral DNA: emergence of new viral sequences in spontaneous AKR/J lymphomas. Proc Natl Acad Sci USA (76): 1677–1681, 1979.
105. Bennett DC, Peachey LA, Durbin H, Rudland PS: A possible mammary stem cell line. Cell (15): 283–298, 1978.
106. Dulbecco R, Henahan M, Bowman M, Okada S, Battifora H, Unger M: Generation of fibroblast-like cells from cloned epithelial mammary cells in vitro: a possible new cell type. Proc Natl Acad Sci USA (78): 2345–2349, 1981.
107. Imada M, Sueoka N: Clonal sublines of rat neurotumor RT4 and cell differentiation. I. Isolation and characterization of cell lines and cell type conversion. Devel Biol (66): 97–108, 1978.
108. Daley MJ: Intratumor maturational heterogeneity within the murine myeloma MOPC-315. Cancer Res (41): 187–191, 1981.
109. Chow DA, Greenberg AH: The generation of tumor heterogeneity in vivo. Int J Cancer (25): 261–265, 1980.
110. Loveless SE, Wang CY, Heppner GH: Mutagenic activity of tumor-associated macrophages (submitted for publication).
111. Weitzman SA, Stossel TP: Mutation caused by human phagocytes. Science (212): 546–547, 1981.
112. Weitzman SA, Stossel TP: Effects of oxygen radical scavengers and anti-oxidants on phagocyte-induced mutagenesis. J Immunol (128): 2770–2772, 1982.
113. Talmadge JE, Starkey JR, Davis WC, Cohen AL: Introduction of metastatic heterogeneity by short-term in vivo passage of a cloned transformed cell line. J Supramol

Struct (12): 227–243, 1979.
114. DeBaetselier P, Gorelik E, Eshhar Z, Ron Y, Katzav S, Feldman M, Segal S: Metastatic properties conferred on nonmetastatic tumors by hybridization of spleen B-lymphocytes with plasmacytoma cells. J Natl Cancer Inst (67): 1079–1087, 1981.
115. Goldenberg DM, Pavia RA, Tsao MC: In vivo hybridization of human tumour and normal hamster cells. Nature (250): 649–651, 1974.
116. Hu F, Pasztor LM: In vivo hybridization of cultured melanoma cells and isogeneic normal mouse cells. Differentiation (4): 92–97, 1975.
117. Lala PK, Santer V, Rahl KS: Spontaneous fusion between Ehrlich ascites tumor cells and host cells in vivo: kinetics of hybridization, and concurrent changes in the histocompatibility profile of the tumor after propagation in different host strains. Eur J Cancer (16): 487–510, 1980.
118. Goldenberg DM, Pavia RA: Malignant potential of murine stromal cells after transplantation of human tumors into nude mice. Science (212): 65–67, 1981.
119. Kerbel RS, Florian M, Man MS, Dennis J, McKenzie IFC: Carcinogenicity of tumor cell populations: origin of a putative H-2 isoantigenic loss variant tumor. J Natl Cancer Inst (64): 1221–1230, 1980.
120. Frost P, Kerbel RS, Tartamella-Biondo R: Generation of highly metastatic tumors in DBA/2 mice. Invasion Metastasis (1): 22–33, 1981.
121. Mantovani A, Giavazzi R, Alessandri G, Spreafico F, Garattini S: Characterization of tumor lines derived from spontaneous metastases of a transplanted murine sarcoma. Eur J Cancer (17): 71–76, 1981.
122. Talmadge JE: Evidence that the process of metastasis is selective and not random. Proc AACR (23): 173 (abstract), 1982.
123. Chambers AF, Hill RP, Ling V: Tumor heterogeneity and stability of the metastatic phenotype of mouse KHT sarcoma cells. Cancer Res (41): 1368–1372, 1981.
124. Neri A, Nicolson GL: Phenotypic drift of metastatic and cell-surface properties of mammary adenocarcinoma cell clones during growth in vitro. Int J Cancer (28): 731–738, 1981.
125. Dennis J, Donaghue T, Florian M, Kerbel RS: Apparent reversion of stable in vitro genetic markers detected in tumour cells from spontaneous metastases. Nature (292): 242–245, 1981.
126. Poste G, Doll J, Fidler IJ: Interactions among clonal subpopulations affect stability of the metastatic phenotype in polyclonal populations of B16 melanoma cells. Proc Natl Acad Sci USA (78): 6626–6630, 1981.
127. Rubin H: Is somatic mutation the major mechanism of malignant transformation? J Natl Cancer Inst (64): 995–1000, 1980.
128. Ludford RJ, Barlow H: The influence of malignant cells upon the growth of fibroblasts in vitro. Cancer Res (4): 694–703, 1944.
129. Ranadive KJ, Bhide SV: Tissue interactions between normal and malignant cells. *In*: Brennan MJ, Simpson WL (eds) Biological interactions in normal and neoplastic growth. Boston, Little, Brown, and Co, 1962, pp 337–354.
130. Stoker M: Regulation of growth and orientation in hamster cells transformed by polyoma virus. Virol (24): 165–174, 1964.
131. Slemmer G: Interactions of separate types of cells during normal and neoplastic mammary gland growth. J Invest Derm (63): 27–47, 1974.
132. Nandi S: Hormonal carcinogenesis: a novel hypothesis for the role of hormones. J Environ Pathol Tox (2): 13–20, 1978.
133. DeOme KB, Miyamoto MJ, Osborn RC, Guzman RC, Lum K: Detection of inapparent nodule-transformed cells in the mammary gland tissues of virgin female BALB/cfC_3H mice. Cancer Res (38): 2103–2111, 1974.
134. Medina D, Shepherd F, Gropp T: Enhancement of the tumorigenicity of preneoplastic mammary nodule lines by enzymatic dissociation. J Natl Cancer Inst (60): 1121–1126, 1978.
135. Simnett JD: Regulation of growth and cell division in the whole organism. *In*: Sherbet GV (ed) Regulation of growth in neoplasia. Basel, Karger, 1981, pp 1–51.
136. Hauschka TS: Methods of conditioning the graft in tumor transplantation. J Natl Cancer Inst (14): 723–736, 1953.
137. Heppner GH: The challenge of tumor heterogeneity. *In*: Bulbrook RD, Taylor DJ (eds) Commentaries on research in breast disease. New York, Alan R. Liss, Inc. 1979, pp 177–191.
138. Makino S: Further evidence favoring the concept of the stem cell in ascites tumors of rats. Ann N Y Acad Sci (63): 818–830, 1956.
139. Chesire PJ: The effect of multiple tumors on mammary tumor growth rates in the C_3H mouse. Br J Cancer (24): 542–547, 1970.
140. Heppner G, Miller B, Cooper DN, Miller FR: Growth interactions between mammary tumor cells. *In*: McGrath CM, Brennan MJ, Rich MA (eds) Cell biology of breast cancer. New York, Academic Press, 1980, pp 161–172.
141. Miller BE, Miller FR, Leith J, Heppner GH: Growth interaction in vivo between tumor subpopulations derived from a single mouse mammary tumor. Cancer Res (40): 3977–3981, 1980.
142. Heppner GH: Tumor subpopulation interactions. *In*: Owens A (ed) Tumor cell heterogeneity: origins and implications. New York, Academic Press, 1982.
143. Riley PA: Control of proliferation of normal and neoplastic cells in culture. *In*: Sherbet GV (ed) Regulation of growth neoplasia. Basel, Karger, 1981, pp 131–198.
144. DeLarco JE, Todaro GJ: Growth factors from murine sarcoma virus-transformed cells. Proc Natl Acad Sci USA (75): 4001–4005, 1978.
145. Horoszewicz JS, Leong SS, Carter WA: Noncycling tumor cells are sensitive targets for the antiproliferative activity of human interferon. Science (206): 1091–1093, 1979.
146. Moody TW, Pert CB, Gazder AF, Carney DN, Minna

JD: High levels of intracellular bombasin characterize human small-cell lung carcinoma. Science (214): 1246–1248, 1981.

147. Nowotny A, Grodsman J: Mixed tumor challenge of strain specific and nonspecific TA3 mouse ascites mammary adenocarcinoma. Int Arch Allergy (44): 434–440, 1973.
148. Miller FR, Heppner GH: Intratumor immunologic heterogeneity. Proc AACR (21): 201 (abstract), 1979.
149. Browning HC: Heterologous and homologous growth of transplants during the course of development of spontaneous mammary tumors in C_3H mice. J Natl Cancer Inst (8): 173–189, 1948.
150. Klein G, Klein E: Conversion of solid neoplasms into ascites tumors. Ann N Y Acad Sci (63): 640–661, 1956.
151. DeWys WD: Studies correlating the growth rate of a tumor and its metastasis and providing evidence for tumor-related systemic growth-retarding factors. Cancer Res (32): 374–379, 1972.
152. Greene HSN, Harvey EK: The inhibitory influence of a transplanted hamster lymphoma on metastasis. Cancer Res (20): 1094–1100, 1960.
153. Milas L, Hunter N. Mason K, Withers HR: Immunological resistance to pulmonary metastasis in C_3Hf/Bu mice bearing syngeneic fibrosarcomas of different sizes. Cancer Res (34): 61–71, 1974.
154. Yuhas JM, Pazmiño NH: Inhibition of subcutaneously growing line 1 carcinomas due to metastatic spread. Cancer Res (34): 2005–2010, 1974.
155. Goldie H, Walker M, Kelley L, Gaines J: Free tumor cell growth in the peritoneal cavity (ascites tumor) of mice bearing subcutaneous tumors. Cancer Res (16): 553–558, 1956.
156. Gorelik E, Segal S, Feldman M: Growth of a local tumor exerts a specific inhibitory effect on progression of lung metastases. Int J Cancer (21): 617–625, 1978.
157. Gorelik E, Segal S, Feldman M: On the mechanism of tumor 'concomitant immunity'. Int J Cancer (27): 847–856, 1981.
158. Miller F: Subpopulation interactions in metastasis. Invasion Metastasis (in press).
159. Poste G, Doll J, Brown AE, Tzeng J, Zeidman I: Comparison of the metastatic properties of B16 melanoma clones isolated from cultured cell lines, subcutaneous tumors, and individual lung metastases. Cancer Res (42): 2770–2778, 1982.
160. Biswas C, Morgan WP, Bloch KJ, Gross J: Collagenolytic activity of rabbit V_2-carcinoma growing at multiple sites. Biochem Biophys Res Comm (80): 33–38, 1980.
161. Poste G, Nicolson G: Arrest and metastasis of blood-borne tumor cells are modified by fusion of plasma membrane vesicles from highly metastatic cells. Proc Natl Acad Sci USA (77): 399–403, 1980.
162. Dvorak HF, Quay SC, Orenstein NS, Dvorak AM, Hahn P, Bitzer AM, Carvallo AC: Tumor shedding and coagulation. Science (212): 923–924, 1981.
163. Sinha AA: Hormone sensitivity and autonomy of tumours. *In*: Stoll BA (ed) Hormonal management of endocrine-related cancer. London, Lloyd-Luke Medical Books, 1981, pp 13–19.
164. Sluyser M: The emergence of hormone-independent cells in hormone-dependent breast cancer. *In*: McGrath CM, Brennan MJ, Rich MA (eds) Cell biology of breast cancer. New York, Academic Press, 1980, pp 173–187.
165. Lee C, Lapin V, Oyasu R, Battifora H: Effect of ovariectomy on serially transplanted rat mammary tumors induced by 7,12-dimethylbenz[a]anthracene. Eur J Cancer Clin Oncol (17): 801–808, 1981.
166. Sinha AA, Blackard CE, Seal US: A critical analyses of tumor morphology and hormone treatments in the untreated and estrogen-treated responsive and refractory human prostatic carcinoma. Cancer (4): 2836–2850, 1977.
167. Isaacs JT, Coffey DS: Adaptation versus selection as the mechanism responsible for the relapse of prostatic cancer to androgen ablation therapy as studies in the Dunning B-3327-H adenocarcinoma. Cancer Res (41): 5070–5075, 1981.
168. Hart IR, Fidler IJ: Cancer invasion and metastasis. Quart Rev Biol (55): 121–142, 1980.
169. Sluyser M, DeGoeij KCJ, Evers SG: Outgrowth of grafts containing different ratios of hormone-dependent and independent mouse mammary tumor cells. Cancer Lett (13): 71–77, 1981.
170. Isaacs JT, Wake N, Coffey DS, Sandberg AA: Genetic instability coupled to clonal selection as a mechanism for tumor progression in the Dunning R-3327 rat prostatic adenocarcinoma system. Cancer Res (42): 2353–2361, 1982.
171. Hager JC, Miller FR, Heppner GH: Influence of serial transplantation on the immunological clinical correlates of $BALB/cfC_3H$ mouse mammary tumors. Cancer Res (38): 2492–2500, 1978.
172. Kiang DT, King M, Zhang HJ, Kennedy BJ, Wang N: Cyclic biological expression in mouse mammary tumors. Science (216): 68–70, 1982.
173. Vaage J: Inherent changes in the in vivo growth characteristics of C_3H/Hc mammary carcinomas. Cancer Res (40): 3495–3501, 1980.
174. Law LW: Origin of the resistance of leukemic cells to folic acid antagonists. Nature (169): 628–629, 1952.
175. Hakansson L and Tropé C: Cell clones with differed sensitivity to cytostatic drugs in methylcholanthrene-induced mouse sarcomas. Acta Pathol Microbiol Scand Section A (82): 41–47, 1974.
176. Tropé C, Aspegun K, Kullander S, Astedt B: Heterogeneous response of disseminated human ovarian cancer to cytostates in vitro. Acta Obstet Gyncol Scand (58): 543–546, 1979.
177. Biörkland A, Hakansson L, Stenstam B, Tropé C, Akerman M: On heterogeneity of non-Hodgkin's lymphomas as regards sensitivity to cytostatic drugs. Eur J Cancer (16): 647–654, 1980.

178. Dexter DL, Spremulli EN, Fligiel Z, Barbosa JA, Vogel R, VanVoorhees A, Calabresi P: Heterogeneity of cancer cells from a single human colon carcinoma. Am J Med (71): 949–956, 1981.
179. Tsuruo T, Fidler IJ: Differences in drug sensitivity among tumor cells from parental tumors, selected variants and spontaneous metastases. Cancer Res (41): 3058–3064, 1981.
180. Sacchi A, Calabresi F, Greco C, Zupi G: Different metastatic potential of in vitro and in vivo lines selected from Lewis lung carcinoma: correlation with response to different bleomycin schedulings. Invasion Metastasis (1): 227–238, 1981.
181. Yung WA, Shapiro JR, Shapiro WR: Heterogeneous chemosensitivities of subpopulations of human glioma cells in culture. Cancer Res (42): 992–998, 1982.
182. Smith HS, Staupfer MR, Hackett AJ: Adriamycin sensitivity of cultured malignant and nonmalignant human mammary epithelial cells (abstract). J Cell Biol (suppl 6), 367, 1982.
183. Lotan R, Nicolson GL: Heterogeneity in growth inhibition by B-trans-retinoic acid of metastatic B-16 melanoma clones and in vivo-selected cell variant lines. Cancer Res (39): 4767–4771, 1979.
184. Hill HZ, Hill GJ, Miller CF, Kwong F, Purdy J: Radiation and melanoma response of B16 mouse tumor cells and clonal lines to in vitro irradiation. Rad Res (80): 259–276, 1979.
185. Leith JT, Brenner HJ, DeWyngaert JK, Dexter DL, Calabresi P, Glicksman AS: Selective modification of the X-ray response of two mouse mammary adenocarcinoma sublines by N,N-dimethylformamide. Int J Rad Oncol Biol Phys (7): 943–947, 1981.
186. Leith JT, Gaskins LA, Dexter DL, Calabresi P, Glicksman AS: Alteration of the survival response of two human colon carcinoma subpopulations to X-irradiation by N,N-dimethylformamide. Cancer Res (42): 30–34, 1982.
187. Leith JT, Dexter DL, DeWyngaert JK, Zeman EM, Chu MY, Calabresi P, Glicksman AS: Differential responses to X-irradiation of subpopulations of two heterogeneous human carcinomas in vitro. Cancer Res (42): 2556–2561, 1982.
188. Berd D, Mastrangelo MJ: Differential sensitivity of two murine leukemia sublines to cytolysis by *Corynebacterium parvum*-activated macrophages. Br J Cancer (44): 819–827, 1981.
189. Dennis JW, Donaghue TP, Kerbel RS: An examination of tumor antigen loss in spontaneous metastases. Invasion Metastases (1): 111–125, 1981.
190. Strzadala L, Opolski A, Radzikowski C, Mihich E: Differential expression of murine leukemia antigen on L1210 parental and drug-resistant sublines. Cancer Res (41): 4934–4937, 1981.
191. Young WW Jr, Hakomori S: Therapy of mouse lymphoma with monoclonal antibodies to glycolipid: selection of low antigenic variants in vivo. Science (211): 487–489, 1981.
192. Miller BE, Miller FR, Heppner GH: Interactions between tumor subpopulations affecting their sensitivity to the antineoplastic agents cyclophosphamide and methotrexate. Cancer Res (41): 4378–4381, 1981.
193. Subak-Sharpe H, Burk RR, Pitts JD: Metabolic cooperation between biochemically marked mammalian cells in tissue culture. J Cell Sci (4): 353–367, 1969.
194. Loewenstein WR: Junctional intercellular communication and the control of growth. Biochim Biophys Acta (560): 1–65. 1979.
195. Salmon SE, Hamburger AW, Soehnlen B, Durie BG, Alberts DS, Moon TE: Quantitation of differential sensitivity of human tumor stem cells to anticancer drugs. N Engl J Med (298): 1321–1327, 1978.
196. Giovanella BC: Experimental chemotherapy of human tumors heterotransplanted in nude mice. Antibiot Chemother (28): 21–27, 1980.
197. Miller BE, Miller FR, Heppner GH: Development of a drug-sensitivity assay for heterogeneous tumors based on growth in 3-dimensional collagen gels. *In*: Chabner BA (ed) Rational basis for chemotherapy. New York, Alan R Liss, Inc (in press).
198. Goldie JH, Coldman AJ: A mathematical model for relating the drug sensitivity of tumors to their spontaneous mutation rate. Cancer Treat Rep (63): 1727–1733, 1979.
199. Luria JE, Delbruck M: Mutations of bacteria from virus sensitivity to virus resistance. Genetics (28): 491–511, 1943.
200. Mulder JH, Smink T, VanPutten LM: 5-Fluorouracil and methotrexate combination chemotherapy: the effect of drug scheduling. Eur J Chem Oncol (17): 831–837, 1981.
201. Gewirtz AM, Cadman E: Preliminary report on the efficacy of sequential methrotrexate and 5-fluorouracil in advanced breast cancer. Cancer (47): 2552–2555, 1981.
202. Cadman EC, Benz C, Voigt J, Heimer R: Enhanced 5-fluorouracil (5FU) nucleotide formation following methotrexate (MTX) is the consequence of increased intercellular phosphoribosylpyrophosphate (PRPP). Proc AACR (20): 258 (abstract), 1979.
203. Roberts D, Peck C: Effect of methotrexate and 1-β-D-arabinofuranosylcytosine on pools of deoxyribonucleoside triphosphates in L1210 ascites cells. Cancer Res (40): 505–510, 1981.
204. Fried J, Perez AG, Doblin JM, Clarkson BD: Cytotoxic and cytokinetic effects of thymidine, 5-fluorouracil, and deoxycytidine on HeLa cells in culture. Cancer Res (41): 2627–2632, 1981.
205. Ritch PS, Occhipinti SJ, Cunningham RE, Shackney SW: Schedule-dependent synergism of combinations of hydroxyurea with adriamycin and 1-β-D-arabinofuranocylcytosine with adriamycin. Cancer Res (41): 3881–3884, 1981.
206. Fodstad Ø, Pihl A: Synergistic effect of ricin in combination with daunorubicin, cis-dichlorodiammine-platinum (II) and vincristine in systemic L1210 leukemia. Cancer Res (42): 2152–2158, 1982.