Tumors: A Mixed Bag of Cells

Most cancerous tumors contain subpopulations of cells with different properties, which helps make cancer hard to treat

It is often said that cancer is hard to cure because it is not one disease but a hundred different diseases. Recent evidence suggests that this may be an understatement, however. At a symposium held last month,* researchers described how a single cancerous tumor might itself be composed of a mixture of cells having different properties. The cell subpopulations can vary in their ability to spread to other sites in the body, in their susceptibility to drug therapy, in the ease with which they provoke or avoid an immune attack, and in their production of chemical "markers" that are sometimes used to detect the presence of particular cancers.

All these variations contribute to the difficulty of treating cancer. A minor subpopulation of drug-resistant cells, for example, can escape the killing effects of chemotherapy and continue to proliferate. According to Paul Calabresi of Roger Williams General Hospital in Providence, Rhode Island, "The crab is the wrong symbol for cancer. They should have used a chimera, a monster in Greek mythology that has a goat's body, a serpent's tail, and a lion's head, and is often shown breathing fire. It is a much fiercer and more dangerous animal than a crab."

The most dangerous tumor cells are those capable of escaping from the original tumor before it is surgically removed and forming new tumors at other sites in the body. This ability to metastasize is not shared equally by all the cells of the initial tumor. As Isaiah Fidler of the Frederick Cancer Research Center in Maryland said at the meeting, "Metastases are not random but result from the selective growth of a subpopulation of cells that preexists in heterogeneous tumors."

In early work Fidler injected tumor cells into the bloodstream of genetically compatible animals. He found that only a small proportion—about 1 percent—survived and formed secondary tumor growths. To show that this situation reflects differences in the metastatic potential of the tumor cells, Fidler and Margaret Kripke of the Frederick Cancer Center cloned individual cells from a malignant melanoma, a skin cancer that frequently metastasizes to the lungs. They then compared the ability of the cloned sublines to form lung metastases with that of a suspension of cells from the original tumor.

If the original tumor consisted of cells equally capable of forming metastases, then the cloned sublines and the tumor cell suspension should have produced the same number of lung tumors when injected into mice. But that was not the case; there were large differences in the numbers produced by the various samples. In other words, some of the original tumor cells had a much greater tendency to spread than others.

The original study was done with a tumor line that had been maintained for more than 20 years. Heterogeneity in that tumor might have been an artifact. But in more recent work, Fidler and his colleagues, including Kripke, Ian Hart of the Frederick laboratory, and George Poste, first at Roswell Park Memorial Institute and now at Smith Kline and French Laboratories in Philadelphia, obtained similar results with several primary mouse tumors as well as with a melanoma that had been newly induced in mice. Fidler concludes, "It becomes very clear that these tumors of recent origin are just as heterogeneous as the tumor passaged for 22 years."

Garth Nicolson of the M. D. Anderson Hospital and Tumor Institute in Houston has been attempting to determine what makes some cancer cells more metastatic than others. He reported in Baltimore on a series of experiments with a mouse lymphosarcoma similar to human lymphosarcoma. The Nicolson group compared the properties of sublines of the mouse tumor, which had been selected for their increased efficiency in producing liver metastases, with those of the parent lymphosarcoma line. The principal differences they found were in the cell surface properties, which is perhaps not surprising because it is through their surfaces that cells contact and interact with one another.

To reach a new site and seed a metastasis, a tumor cell must travel through the bloodstream, where it may be attacked and destroyed by any of a number of immune cells, including killer T cells, natural killer cells, or macrophages. Such an attack would presumably be directed against some exposed component of the target cell surface. According to Nicolson, treatment that impairs macrophage activity abolishes the metastatic difference between the parent lymphosarcoma and the more dangerous sublines, with the parent line producing as many metastases as the sublines. Inhibiting killer T cell and natural killer cell activities had no effect on the relative metastatic potentials of the cell lines. Nicolson says, "The message is that there seems to be a response against the parental tumor and it seems to be mediated by macrophages."

Attack by the macrophages on the parental lymphosarcoma cells may be elicited by gp (glycoprotein) 70, a constituent of RNA tumor viruses that is often found on the surface of mouse tumor cells. Nicolson reports that there is an inverse relationship between the amount of gp 70 in the tumor cell membrane and the cell's ability to metastasize. He hypothesizes that the glycoprotein might constitute all or part of the target for the macrophages.

Cancers generally have preferred metastatic sites. Breast cancer, for example, tends to spread to the bone or brain, and colon cancer to the liver. Cell surface molecules may help guide metastatic cancer cells to their preferred locations. Nicolson says that gp 70 does not help the lymphosarcoma cells to colonize liver, but that another cell surface component, similar or identical to a substance usually found only on fetal liver cells, does appear to be involved. Antibodies directed against the fetal antigen block metastasis by the maligant lymphosarcoma line and prolong the survival of mice injected with the cells.

Not only may the cells of a tumor be heterogeneous with regard to their ability to spread, they may also vary in their susceptibility to drugs or radiation used to treat the tumor. For example, Claes Tropé of the University Hospital of Lund, Sweden, divided mouse tumors

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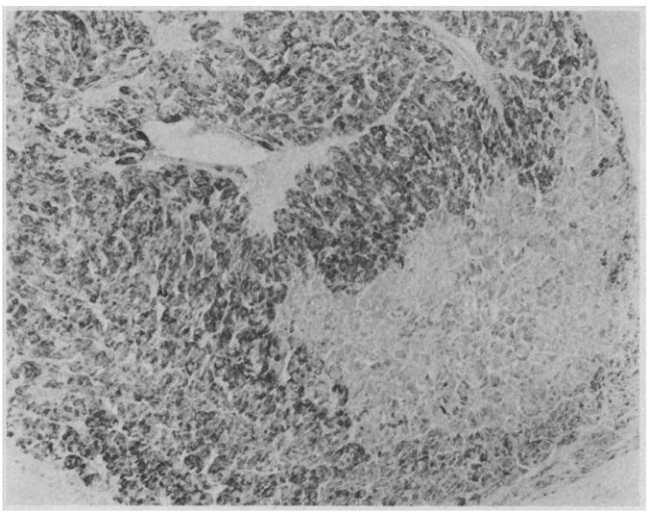

Tumor cell heterogeneity

This human thyroid carcinoma contains cells that make the hormone calcitonin (dark-staining areas) and cells that have lost that ability (light areas). [Source: Adapted from D. L. Trump, G. Mendelsohn, and S. B. Baylin, New Eng. J. Med. *301, 253 (1979)]*

into quarters, made cell suspensions of the pieces, and showed extensive diversity in the responses of the individual samples to the chemotherapeutic drugs.

In addition, Calabresi has cloned cells from each of two regions of a human colon cancer that were very dissimilar in appearance. The two clones responded very differently when tested with drugs. This type of heterogeneity may help to explain why combination chemotherapy has often succeeded in curing or controlling cancers in situations where a single drug has failed.

The two clones prepared by Calabresi also differed in the production of carcinoembryonic antigen (CEA), with one making large quantities of the material and the other very little. Although CEA in the blood is not an absolutely specific indicator of the presence of colon cancer, it is often measured either to detect the initial cancer or its recurrence after surgery. Heterogeneity in CEA production has implications for its use as a marker. Calabresi notes, "If a tumor derived from low-producer cells is growing, you could miss early detection of the tumor."

Still another type of tumor cell heterogeneity was described by John Isaacs of Johns Hopkins University School of Medicine. Some cancer cells will not grow unless they are stimulated by a hormone. This is often true for breast cancer cells, which may require estrogen, for example, and prostate cancer cells, which may depend on the presence of androgen.

Human prostate cancer is frequently treated either by castration to remove the source of the androgen or by administration of the synthetic female hormone diethylstilbestrol to block the action of androgen on its target cells. But according to Isaacs, most patients eventually relapse because the tumor becomes androgen-insensitive and begins to grow again.

Experiments with an animal model for prostate cancer suggested to Isaacs that the tumors already contain a few androgen-insensitive cells at the time of detection. If that is the case for human prostate cancers as well, there are important clinical implications. Simply removing androgen or blocking its effect can never cure the cancer. The androgen-insensitive cells will continue growing. Isaacs suggests that the initial therapy should include some additional treatment, such as radiation or chemotherapy, to knock out the resistant cell population.

This theme was sounded by other participants at the symposium, including Calabresi. The current practice in treating many cancers is to try one agent to obtain regression of the tumor. Then if the patient relapses, another agent is used. However, this strategy allows the emergence of large tumors composed of cells resistant to the initial agent and may permit the development of still more heterogeneity.

Calabresi suggests that it may be better to use a combination of therapies, not necessarily all at once, but in a sequence that allows the patient some time to recover from the side effects of a particular treatment without allowing enough time for the residual tumor cells to form a large tumor. The problem is choosing the right agents and the appropriate sequence for their administration.

Most current drug screening procedures do not take into account the heterogeneous nature of tumor cell populations. Sometimes tumor samples from a patient are cultured and tested for their susceptibility to drugs. But this may not produce reliable information. If the resistant population constitutes a small fraction of the total, it will be difficult to tell whether the drug left surviving cells. This will be particularly dangerous for the patient if the survivors are the ones with high metastatic potential.

Some type of multistep screen may be required to determine the best sequence of therapies. In one such attempt, Calabresi implanted cells from the two colon cancer clones in the flanks of nude mice, which cannot reject such tissue grafts. He then irradiated the resulting tumors. Samples of the surviving cells were cultured and tested for their susceptibility to various drugs.

How tumors become heterogeneous in composition is not completely understood. Although some tumors may be heterogeneous to start with because they arise from several cells that have undergone malignant transformation, others that are known to be clones derived from a single cell are also composed of cells having different properties.

At the Baltimore meeting several investigators presented evidence that tumor cells are more likely to undergo gene changes than are normal cells. Peter Nowell of the University of Pennsylvania School of Medicine said, "The fundamental thesis is that tumor cells are in many instances more genetically unstable than normal cells. Heterogeneity is produced because they undergo more and more mutations."

The chromosomes of tumor cells even look abnormal, according to Avery Sandberg of Roswell Park Memorial Institute. Moreover, the chromosome numbers of cells within the same tumor may vary tremendously, although at least some cancers have characteristic marker chromosomes found in all cells. The most famous example of such a marker is the Philadelphia chromosome, in which a portion of chromosome 22 has been attached to chromosome 9 in the white blood cells of patients with chronic myelogenous leukemia. More recently, John Minna and his colleagues at the National Cancer Institute identified a marker for small-cell carcinoma of the lung. They found that a portion of the small arm of chromosome 3 is deleted in 20 different lines of cells cultured from human small-cell carcinomas.

Visible chromosome changes may be related to alterations in the malignant behavior of a tumor. Sandberg cites as an example the meningioma, a tumor of the brain lining, which is usually localized and unlikely to spread. Occasionally, however, a meningioma will develop into a sarcoma, a highly invasive and lethal cancer.

The cells of ordinary meningiomas are characterized by the loss of chromosome 22. Because the cells of the invasive

tumor have many more abnormalities, Sandberg concludes, "The addition of other changes is the key factor in changing the biology of the tumor."

Large chromosome changes are also associated with an increase in the lethality of the prostate tumor studied by Isaacs. This tumor, when induced in rats, usually doubles in size in about 20 days, but in an occasional animal the doubling time is only 3 days. The fast growing tumors contain areas of cells that are more primitive in appearance than the original tumor cells. The aberrant cells lack androgen receptors and have almost twice as many chromosomes as the slower growing cells. "The parental line," Isaacs says, "has given rise to a genetically different line."

More subtle genetic variability can also be observed in tumor cells. Ruth Sager of Harvard Medical School reported that the mutation rate of tumor cells is greater than that of normal ones. She found that CHEF cells (Chinese hamster embryo fibroblasts) required 1 year to show a 100-fold increase in resistance to the drug methotrexate, while tumor cells increased their resistance at four times that rate. Sager's conclusion is that "Cancer is evolution speeded up."

Finally, Fidler has evidence that genetic instability and tumor progression from benign to malignant are related. With Maria Cifone, he has found that highly metastatic clones isolated from four different mouse tumor systems have a higher rate of spontaneous mutation, as measured by the development of resistance to the drugs ouabain and thioguanine, than less malignant clones.

In addition, Poste and his colleagues have evidence that the cells of clones of melanoma cells can undergo rapid changes in metastatic potential when maintained in culture or in the living animal. Cell lines composed of a mixed population of melanoma cells do not show these changes, however. Poste suggests that interactions between the different tumor cells somehow help to maintain a stable population.

Further evidence that the heterogeneous cells of a tumor interact with one another was described by Gloria Heppner of the Michigan Cancer Foundation. For example, they can influence the drug sensitivity or rate of growth of other cells in the tumor. Heppner says, "Tumors are more complex than a roll call of their subpopulations, however many there are, may suggest."

Taken together, these studies lead to a chilling hypothesis, namely, that cancer therapy that disturbs the equilibrium of a tumor cell population may actually favor what Poste and Fidler call "the relentless emergence of new subpopulations with enhanced metastatic capacities."

Large changes in the genome, including visible alterations in chromosome numbers or appearance, probably reflect late changes in the tumor cell. Several symposium participants, including I. Bernard Weinstein of Columbia University's College of Physicians and Surgeons, Paul Ts'o of the Johns Hopkins School of Hygiene and Public Health, and Paul Nettesheim of the National Institute of Environmental Health Sciences, emphasized that the development of a malignant tumor is not a simple one-step change but requires several steps. Weeks, months, or even years may be required for a cell to progress from the first triggering event to a tumor with metastatic potential. Consequently, the initial changes in the DNA of the tumor cells may have been much more subtle than the major chromosomal changes seen in fully developed tumors, assuming, of course, that a DNA change is involved.

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At least some tumor cells are not permanently locked in the malignant state, which suggests that they have not undergone a DNA change. G. Barry Pierce of the University of Colorado School of Medicine described work by his group and that of Beatrice Mintz of the Fox Chase Institute for Cancer Research showing that teratocarcinoma cells, which are highly malignant in mice after birth, develop normally when placed in mouse embryos.

Gene changes, whether induced by viruses, chemicals, or radiation, are thought to be involved in the development of many, perhaps most, other cancers, however. Susan Astrin of the Fox Chase Institute for Cancer Research presented intriguing evidence, obtained in collaboration with William Hayward's group at Rockefeller University, on how one virus might induce cancer by altering gene expression. Their findings suggest that avian leukosis virus (ALV) causes lymphomas and other cancers in birds by turning on a particular cellular gene, the *myc* gene.

Many investigators think that the inappropriate expression of a normal cellular gene, such as a gene that ought to be active only during the embryonic period, when rapid cell division is called for, and not in mature cells, can lead to cancerous transformation of a cell. This, incidentally, would be consistent with suggestions that cancer cells often behave like immature, undifferentiated cells. For example, Stephen Baylin of Johns Hopkins University School of Medicine presented evidence that the most metastatic cells from a human thyroid tumor are the least differentiated. The tumor Baylin is studying, a medullary carcinoma of the thyroid, arises from a cell that secretes the hormone calcitonin, but the tumors of patients with the least favorable prognoses contain patches of cells that no longer produce the hormone, an indication that they have lost one of their differentiated functions. Metastatic tumors also produce little of the hormone.

The Astrin and Hayward groups have evidence that the human analog of the *myc* gene is turned on in the cancerous white blood cells of some patients with leukemias or lymphomas, but not in white blood cells from normal individuals. This does not mean that a virus causes the human cancers or even that expression of the *myc* gene is behind the malignant changes seen in the cells. The gene expression itself might be the result of some other change that made the cells cancerous.

The existence of heterogeneity in tumor cell populations, however it originates, has many implications for both the study and the treatment of cancer. Researchers cannot assume, for example, that what they learn about cells from a primary tumor and their responsiveness to a therapy also applies to metastatic lesions in the same patient or even to cells taken from another region of the same tumor. While this clearly makes the design of appropriate therapies more difficult, research on how tumor cell subpopulations differ from one another may finally provide the information needed to tame the fierce chimera.

—JEAN L. MARX