RESISTING CANCER CHEMOTHERAPY

Researchers are struggling to learn how cancer cells survive in the presence of lethal drugs. The aim is to prevent resistance.

By JOANNE SILBERNER

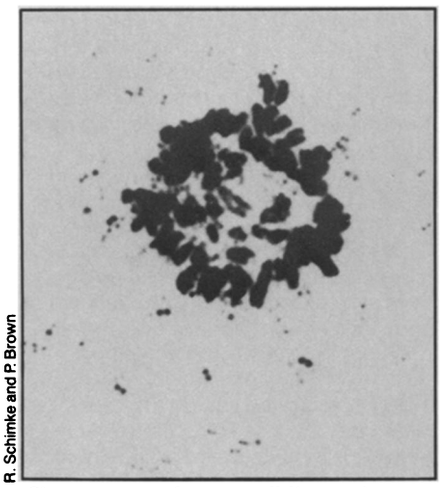
The ideal anticancer drug would be an agent that could kill cancer cells without harming normal cells, and one that cancer cells couldn't neutralize. A detailed understanding of the second component — how cancer cells resist chemotherapy or acquire an immunity to it after exposure — is crucial if cancer researchers are to develop successful countermeasures.

When some of the leaders in the field gathered in Washington, D.C., recently for a symposium on cancer drug resistance, what emerged was a picture of a problem that can arise in any cancer, with at least part of the blame placed on some fancy genetic changes and a specific cell membrane protein.

Clinically, all cancers are, or have the potential to become, resistant to chemotherapy, says oncologist Paul V. Woolley III of Georgetown University in Washington, D.C. With potentially curable cancers, the chances of survival go down dramatically with each recurrence — the second, third or fourth time around, the cancer is much less responsive to chemotherapy, says Woolley. "From a clinical point of view, the emergence of resistance, if it's not there to begin with, is a universal problem."

Resistance, he says, is either inherent in the cancer cell, or acquired when the cell is exposed to chemotherapy. These cells initially started out as normal cells. In the inherent form of resistance, this origin can be the source of the problem; some cancer cells retain whatever it is that allows the normal cell to persist in the presence of a toxic drug.

Colon cells, for example, are regularly exposed to toxins in digested food passing through the intestine, and are more resistant than other normal cells to conventional chemotherapeutic agents. The cancers that arise from colon cells tend to maintain that property. "Despite a quarter century of drug testing, we have made only at best modest gains in the [chemical] treatment of colon cancer," Woolley said at the symposium, which was organized by Georgetown and sponsored by Bristol-Myers Co.



R. Schimke and P. Brown

Chromosomes from a cell line resistant to the anticancer drug methotrexate. The black dots are extrachromosomal DNA pieces thought to include extra copies of a gene coding for the target of methotrexate; extra target protein enables the cell to survive in the presence of the drug.

The other type of resistance, the acquired form, has its roots in the cancer cell's ability to change, which it has already demonstrated in its metamorphosis from a normal cell. The development of drug resistance is just one more change; the key to the puzzle is figuring out how that change occurs.

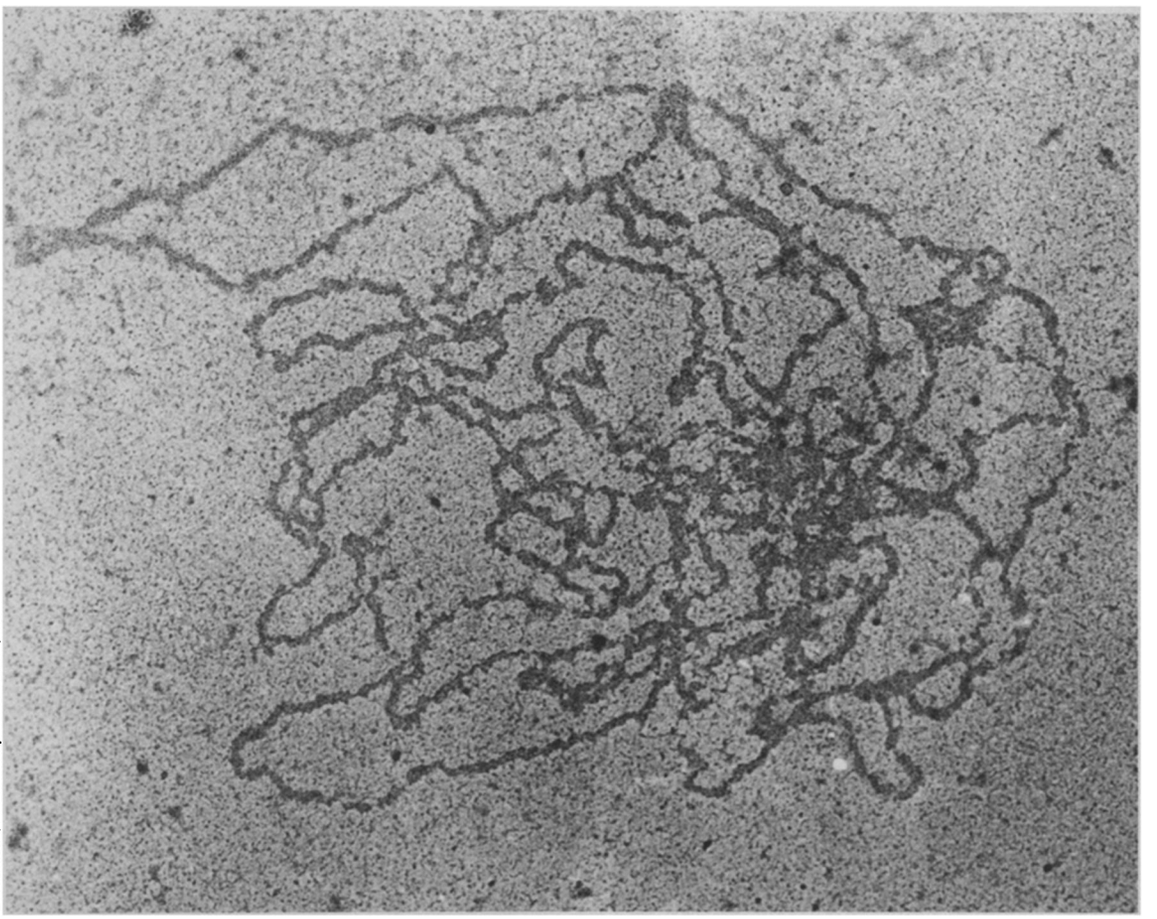
Robert T. Schimke of Stanford University says that at least part of the answer is the overproduction of a gene or genes whose protein products enable the cell to withstand a chemotherapeutic onslaught. Such gene amplification, he says, is "one of the most common types" of cancer drug resistance.

Schimke has seen an example of this in his studies of resistance to the cancer drug methotrexate. The drug works by inhibiting the activity of an enzyme, dihydrofolate reductase (DHFR), that is instrumental in the synthesis of a DNA precursor necessary for cell reproduction.

Multiple copies of the DHFR gene have been found in tumor cells of some methotrexate-resistant patients, enabling the production of above-normal amounts of DHFR. "You have more enzyme than you have methotrexate within the cell," he says. "So even though you've inhibited most of the enzyme [with methotrexate], there's always going to be some free enzyme that can carry out the reaction." It's too early to say whether such amplification accounts for all methotrexate resistance, Schimke says.

The gene amplification process does not work by killing sensitive cells, thereby promoting the growth of preexisting cells that are inherently resistant to the drug. Such selection was once the standard explanation for how cancers dodge chemotherapy. Instead, methotrexate itself "is in fact generating resistance" by causing a change in genetic material, he says. In that sense, chemotherapy can be considered mutagenic. "I suspect the very process [chemotherapy] that's leading to the death of these [cancer] cells is in another form leading to these cells' resistance," he says.

In addition, other processes that block DNA synthesis — other chemicals, ultraviolet light, oxygen deprivation — can amplify genes spontaneously, his labora-



An electron micrograph of a single piece of extrachromosomal DNA. The DNA, from a drug-resistant cell, likely includes resistance-conferring genes.

B. Hamkalo, R. Schimke/PNAS Vol. 82, No. 4

tory has shown.

Schimke and his colleagues suggest three possible sources for the extra genetic material — from dying cells, from unequal distribution of genetic material during cell division, or from overreplication of a DNA segment. "I suspect all three occur," says Schimke. By far the most frequent, he suggests, is partial overreplication.

Asked why malignant cells have the ability to amplify genes, Schimke replies, "I basically have no answer." The talent may be related to the process of cancer itself, Schimke suggests — whatever it is that causes a normal cell to lose its growth constraints may result in the ability of the unrestrained cell to reproduce its DNA unevenly.

A second resistance mechanism, which may or may not be related to gene amplification, involves a protein discovered by Victor Ling of Ontario Cancer Institute in Toronto in the mid-1970s. Putting the gene for the protein into nonresistant cells, he found, gives them the ability to survive in the presence of otherwise lethal drugs.

Ling discovered the protein, called p-glycoprotein, in mammalian cell lines and tumor cells exhibiting resistance to many structurally unrelated drugs. These cells, Ling found, had high levels of p-glycoprotein in their membranes, and the higher the p-glycoprotein the greater the degree of cell resistance.

The function of the protein is unknown. Because it is structurally similar to proteins that ferry other molecules in bacteria, Ling suggests that p-glycoprotein may confer resistance by ejecting absorbed cancer drugs from the cell before they can kill it.

There may be other factors at work. June Biedler of Memorial Sloan-Kettering Cancer Center in New York studied 12 cell lines from mouse, human and hamster cancers and found that not only p-glycoprotein but also another protein, sorcin, and a cell membrane receptor, epidermal growth factor, were present in high concentrations. While she and her colleagues observed p-glycoprotein and sorcin gene amplification, the increased levels of epidermal growth factor are due not to gene amplification but to increased protein production by its gene, she says.

The goal of all this effort is to figure a way around the resistance process. Neither the p-glycoprotein nor the gene amplification work has gotten to that point yet; most current clinical approaches involve hit-or-miss chemotherapy strategies to counter resistance. But one approach based on a possible end result of either process — excess protein production — is already up to the human trial stage. Leonard C. Erickson of Loyola University Medical Center in Maywood, Ill., is working on resistance to certain DNA-damaging drugs. Erickson's work is based on the hypothesis that some cancer cells become resistant by producing higher levels of a repair enzyme that fixes up the damage caused by the drugs. Something that interrupts the repair mechanism would make the cell more sensitive to the anticancer agent.

Erickson and his colleagues have tried treating the cancer cells first with streptozotocin, a naturally occurring product first isolated from fungi. The streptozotocin attaches to a repair enzyme, making the cells more sensitive to the DNA-damaging agent. In the laboratory, the one-two punch worked on cells that were resistant to the cancer drug alone; the streptozotocin increased the number of cells killed by a factor of 1,000 to 10,000. Loyola has just begun toxicity testing in 10 patients with advanced gastrointestinal cancer. It is much too early to judge the efficacy of the treatment, Erickson says.

Clearly something is needed to counter resistance. Currently available drugs, says Georgetown's Woolley, "have allowed enormous therapeutic gains, but we're currently on a plateau. There's a widespread feeling among people in the cancer field that further treatment gains will be a result of a much more detailed understanding of neoplastic [cancer] cells and an understanding of the mechanism by which the cells can adapt to and resist neoplastic drugs." □