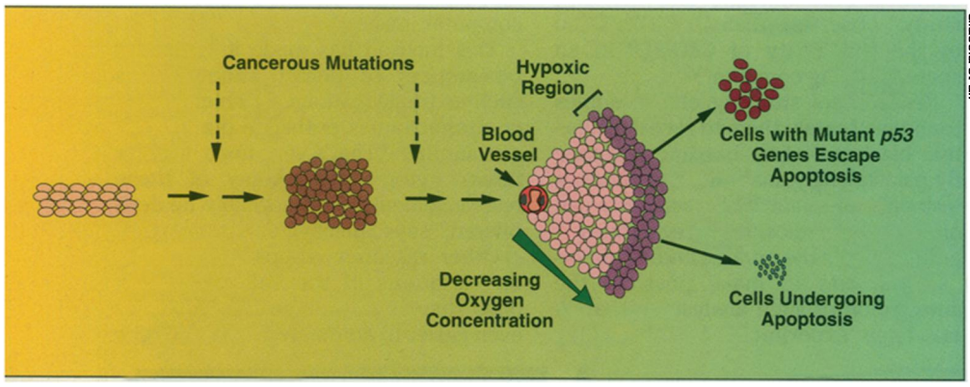
Only the Strong Survive

The evolution of a tumor favors the meanest, most aggressive cells

By LISA SEACHRIST

"What doesn't kill you, makes you strong," the saying goes. But this aphorism's meaning takes a deadly turn when the subject is cancer.

Physicians can attest that many patients eventually succumb to cancer long after seemingly successful treatment. Presumably, a few of the victims' cancer cells somehow evaded anticancer drugs and radiation. Much later, these surviving cells resurfaced as stronger, more aggressive renditions of their earlier selves. The tumors they then formed could resist drugs and radiation. Researchers conclude that such relapses occur because the cells that persist despite anticancer treatments had an enormous competitive advantage over the other malignant cells in the tumor.



Giaccia *et al.*

The Stanford team speculates that mutations in normal cells (left) result in overgrowth. In response to DNA damage, the cells produce the protein p53. As a tumor increases in size and areas farthest from blood vessels become hypoxic, the cells undergo apoptosis. The only cells to survive and multiply are those with mutations in both copies of the p53 *gene.*

More puzzling to physicians are the many tumors that resist even the first anticancer treatments they confront.

Research now indicates that the very stresses cancer cells endure, even before they are detectable, promote the emergence of stronger, more aggressive tumors. California researchers have found that the low-oxygen environment in the center of a tumor specifically fosters cells that have lost their "emergency brake" against uncontrolled cell growth. Their findings also explain why over 50 percent of all solid tumors harbor mutations in the *p53* gene: It is the blueprint for this emergency brake.

"Mutations in *p53* give tumor cells a survival advantage," says study leader Amato J. Giaccia of Stanford University School of Medicine. "Our work indicates that the microenvironment surrounding a tumor may play a significant role in determining how aggressive it ultimately becomes."

The protein coded for by the *p53* gene plays a pivotal role in preventing cancerous cells from growing unchecked. Under normal circumstances, cells do not produce this p53 protein. But exposure to damaging stresses, including heat, starvation, toxic chemicals, and ionizing radiation, causes cells to synthesize it rapidly.

The p53 protein has different effects in different types of cells. In fibroblasts, which make up connective tissues, p53 accumulates and halts cell growth. By grasping specific areas of DNA, p53 triggers the cells to make proteins that stall the process of cell division—presumably to allow time for the cell to repair its damaged DNA.

In other cells, such as white blood cells, p53 sets off another cascade of reactions. Rather than simply arresting cell growth, the protein activates a cell suicide program called apoptosis. "In essence, the cell is programmed to die rather than risk propagating the potentially cancer-causing DNA damage," says Giaccia.

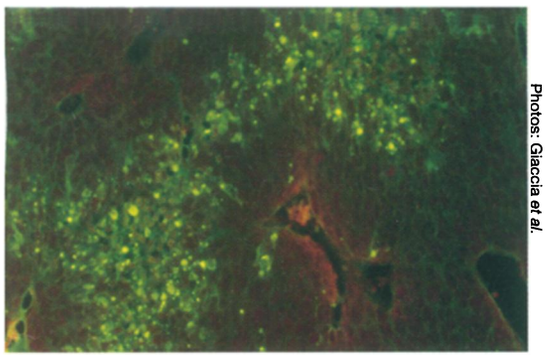
A person inherits a maternal and a paternal copy of the *p53* gene, each providing a blueprint for the p53 protein. As long as a cell can reliably produce p53 protein from one of these genes, it can check overzealous cell growth either through growth arrest or apoptosis. A cell that suffers genetic damage to both copies of *p53* can no longer produce any active protein. As a result, the cell fails to apply the brakes when it has suffered mutations and developed malignant characteristics. Because cells need p53 to prevent cancer, scientists call it a tumor suppressor.

In attempting to understand what types of stresses spur p53 production, Giaccia and Stanford collaborator Thomas G. Graeber noted in the September 1994 MOLECULAR AND CELLULAR BIOLOGY that a low-oxygen environment, or hypoxia, induces p53 synthesis. Because a tumor lacks normal blood vessels, oxygen does not diffuse from the bloodstream to the tumor's innermost parts. The tumor's core becomes littered with pockets of hypoxia filled with dead and dying cells.

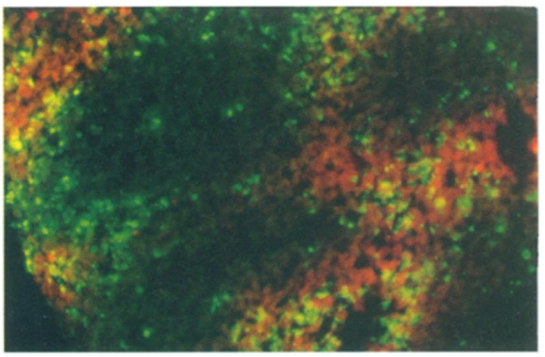
Knowing that hypoxia slows cell division, the researchers assumed that the accumulating p53 made the cell stall in the middle of its growth cycle until it had enough oxygen to resume dividing. However, they also found that hypoxia arrested cell growth even in the absence of functional p53 protein.

"That's when things started to get really interesting," says Giaccia. "If the p53 didn't arrest cell growth, why was the cell producing so much of the protein?"

Clinical researchers have known for



Photos: Giaccia *et al.*



Left: This tissue section shows a tumor that surrounds blood vessels. The green fluorescent dye marks areas of apoptosis. The areas farthest from the blood vessels show the most apoptosis.

Right: Hypoxic areas, indicated by red fluorescent dye, correspond to regions of apoptosis, marked with green dye.

some time that tumors with many hypoxic regions are extremely difficult to kill with radiation or chemotherapy. Radiation inflicts direct damage on DNA, but without oxygen to act as a fixative, the damage may not be permanent or lethal. As for chemotherapy, researchers assume that because toxic drugs usually target actively dividing cells, hypoxic cells gain immunity as a result of arrested cell growth.

Previous research also indicated that tumor cells harboring mutations in both copies of their *p53* genes could more readily escape anticancer therapies. After finding that the p53 protein didn't seem to have any connection to growth arrest in hypoxic cells, the Stanford researchers posited that p53 in hypoxic cells was performing its other role—triggering apoptosis.

The team studied cultures of rat fibroblasts that had begun to take on cancerous characteristics. They exposed some to hypoxic conditions and let the others grow in normal oxygen concentrations. As they report in the Jan. 4 NATURE, 85 percent of the hypoxic cultures initiated their suicide programs and died, whereas only 10 percent of the oxygenated cultures succumbed to apoptosis. When the group tried the same experiment in fibroblasts with two defective copies of the *p53* gene, all of the cells toughed out the hypoxic conditions.

Graeber concludes that "p53 is essential for cells to undergo apoptosis in a hypoxic environment. The way p53 is involved in the stress responses following radiation and hypoxia is quite different."

All of this information came from cells grown in culture dishes. To test the relevance of their results to cancers in the body, the researchers examined sections of mouse tumors. They discovered that the areas in which they found the most apoptosis were indeed the most hypoxic and that tumors with mutations in both *p53* genes had far fewer areas succumbing to apoptosis than tumors with normal *p53* genes.

"If the cells dying had functional p53, then hypoxia is probably playing a strong selective role in the tumor," says Giaccia. "Only cells [lacking p53] will survive and grow."

Giaccia and his team tested their theory by mixing together cells from normal rat fibroblast cultures and from *p53* double-mutation cultures and exposing the cells to rounds of hypoxia. Even though there was only 1 mutant cell for every 1,000 normal cells at the beginning of the experiment, after seven hypoxia treatments the cultures contained more mutant cells than normal ones.

"This may be a dangerous step in tumor progression, and one more way that tumors have of gaining a selective advantage," says Judah Folkman of Children's Hospital and Harvard Medical School in Boston. "It is surprising that p53 plays so many roles in the cell."

Bert Vogelstein, a Howard Hughes Medical Institute researcher at Johns Hopkins University in Baltimore, notes that the work could be very important because "these results get at the biology of what is happening inside the tumor and not just results from a Petri dish."

From their experiments, the Stanford group devised a model of how normal cells progress to aggressive tumors. The tumor starts as a single cell that suffers a mutation in a gene; that cell then initiates a small tumor. As the tumor continues to grow, new mutations crop up in the cell's DNA. As long as the tumor remains no more than 100 to 150 micrometers in diameter, its cells can freely use the oxygen found in the surrounding tissue.

Once the tumor exceeds that size, cells at its center begin to die via p53-mediated apoptosis. Conditions now spur the tumor cells to establish new blood vessels that can increase the oxygen supply to the tumor. In a process known as angiogenesis, disorganized and leaky blood vessels grow toward the tumor in order to nourish it. While this temporarily solves the tumor's oxygen deficit, Folkman notes, the leaky plumbing permits blood plasma to empty into the areas between the cells. Inside the tumor, the leakage increases pressure, which closes off blood vessels and exacerbates the hypoxia.

Trapped without oxygen and surrounded by toxic debris from dying cells, cells inside the tumor face the p53-ordained death program. Only cells with mutations in both copies of the *p53* gene can escape—and these survivors multiply rapidly into a more aggressive cancer.

"Upon first analysis, I found these very sobering results," says study author Scott W. Lowe, now at Cold Spring Harbor (N.Y.) Laboratory. "Tumor progression leading to selection against apoptosis helps explain why these tumors are resistant to almost all of our drugs."

Most chilling in Lowe's mind was the fact that the conditions that lead to such a selection take place while the cancer is still very small and probably undetected, so physicians are often battling these aggressive cancers from the outset. "Because almost all the chemotherapeutic drugs kill through very similar mechanisms to hypoxia, we may need to totally change the type of drugs we use," says Lowe.

Upon further consideration, Lowe found "a lot to be optimistic about, because the discovery has brought apoptosis and angiogenesis researchers into collaboration." Vogelstein agrees, saying that the Stanford team's discovery will probably lead to better understanding of basic cancer biology and tumor progression, even if it doesn't lead to new pharmaceuticals.

To understand the biology, Graeber notes, the team needs to confirm that the relationship between hypoxia and apoptosis holds in human tumors. Giaccia intends to explore the signals that tell the cell it has become hypoxic and that stimulate p53 production. Lowe plans to examine how p53 has such a variety of effects. While none of these investigations promises to cure cancer in the next 5 years, Lowe notes that "you have to know where the problem is before you can fix it."

Scientists may not need to understand a process fully to attack it, however. At least one new agent is aimed solely at the hypoxic regions of tumors. Working with the knowledge that hypoxic cells can withstand radiation and chemotherapy treatments, J. Martin Brown from Stanford developed a drug known as tirapazimine, which kills only hypoxic cells, leaving well-oxygenated ones unscathed. The drug has shown so much promise that the French pharmaceutical company Sanofi/Winthrop has begun a large clinical trial in patients with advanced lung cancer.

While opportunity may have delivered tirapazimine, Brown finds promise in Giaccia's work. He notes, "Knowing the mechanism of how hypoxia leads to drug resistance can only help people develop better chemotherapeutic agents." □