TUMOR ANGIOGENESIS

Gene Expression Patterns Identified

Researchers have obtained the most detailed sketch yet of how cancerous tumors secure the blood supplies that nourish their growth. On page 1197, a team led by Bert Vogelstein and Kenneth Kinzler of Johns Hopkins University School of Medicine reports the results of a large-scale comparison of the genes expressed in the blood vessels of human colon cancers and of normal colon tissue. They've found that the gene expression patterns of the two types of vasculature are distinctly different.

Researchers had previously identified a few "markers" that differ between normal blood vessels and the presumably newly formed vessels of tumors, but nothing on the scale seen by the Hopkins workers, who picked up differences in the activity of nearly 80 genes. "It is an important paper," says Erki Ruoslahti of the Burnham Institute in La Jolla, California, who studies tumor vasculature. "I found it surprising that there would be so many markers with such big differences."

Ruoslahti and other researchers interested in developing new cancer therapies are intrigued by the results. Philip Thorpe of the University of Texas Southwestern Medical Center in Dallas notes that the work could point the way to compounds that home in on the protein products of genes that are overexpressed in tumor vessels, in hope of shutting off the growth of blood vessels the tumor needs to survive. Angiogenesis researcher Judah Folkman of Harvard Medical School in Boston, who pioneered the idea of killing tumors by targeting their blood vessels, describes the paper as a "landmark."

Ring of difference. The cells immediately surrounding the purple-stained blood vessels in this colorectal tumor are living (red arrows), while those farther away are dead (black arrows).

Before achieving that landmark, the Hopkins team had to overcome a serious obstacle—learning how to isolate pure populations of the endothelial cells that form blood vessels. These cells are only one of many cell types found in tumor and normal tissues, and they are present in relatively small amounts. The task of sifting them out fell to postdoc Brad St. Croix, who spent 2 years on the problem. He eventually succeeded by using a protein called P1H12, which occurs mainly on endothelial cells, as a handle to separate out the cells. Angiogenesis researcher Noel Bouck of Northwestern University Medical School in Chicago describes the feat as a "tour de force."

Once the isolation method was worked out, Kinzler, Vogelstein, and their colleagues used a technique developed in their lab about 5 years ago to compare the gene expression patterns of endothelial cells from colon cancers and from normal colon tissue from the same patients. Called SAGE, for serial analysis of gene expression, the method involves making DNA copies of the messenger RNAs present in each type of cell and then combining up to 50 short nucleotide "tags" cut from each of these cDNAs into longer DNAs for sequencing (*Science*, 20 October 1995, pp. 368 and 484).

The Hopkins team found some 100,000 sequence tags, representing more than 32,500 active genes, in both normal and tumor endothelial cells. The expression patterns of 79 of these genes were significantly different in the two cell types: 46 were substantially more active, and 33 were less active, in tumor cells than in normal colon endothelial cells. What's more, the researchers found similar expression patterns for many of the same genes in vessels from other tumors, including lung, brain, and metastatic liver cancers. "It's clear that the tumor vasculature is different from normal vasculature," says Douglas Hanahan of the University of California, San Francisco.

Many of the genes expressed at higher levels had not been previously identified. But others are known genes, involved in such activities as forming or remodeling the extracellular matrix—necessary events in the formation of the new blood vessels thought to be present in growing tumors. In fact, the results indicate that the tumor vasculature is very similar to newly forming blood vessels elsewhere, such as in healing wounds. "Virtually all the genes expressed in the tumor vasculature were expressed in neovasculature that was not neoplastic," Vogelstein says.

In theory, this could pose problems for drug development. Compounds that block the overactive tumor vessel genes might also disrupt normal blood vessel growth, or angiogenesis. But, says Kinzler, "I don't think it means that there won't be good targets for therapy." Others agree. Hanahan notes, for example, that early studies of antiangiogenesis drugs haven't picked up any particular problems with normal angiogenesis, which in the adult is mainly confined to wound-healing and the growth of the uterine lining

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ScienceScope

Horseshoe Haven Scientists are uncertain if horseshoe crabs are being overharvested (see p. 1122), but the federal government is moving to set up a refuge for the animals anyway. Commerce Department officials last week proposed a 6164-square-kilometer sanctuary off Delaware Bay, believed to host the largest horseshoe crab population on the Atlantic seaboard.

Harvesters collected an estimated 3 million crabs in 1998 for use as bait—up significantly from past years. Despite a lack of rock-solid evidence for population declines, however, Commerce officials want to end harvesting in the new refuge by 30 October. "The last thing any of us wants is to see these creatures wind up on the fished-out stocks list," Commerce Secretary Norman Mineta said at an 8 August press conference. "We look at it as a risk-averse approach," adds biologist Paul Perra of the National Marine Fisheries Service. And conservationist Glenn Gauvry, director of the Ecological Research & Development Group in Milton, Delaware, says that "protecting spawners off the Delaware Bay is very sensible. It's the heart of the population."

A formal notice of the proposal, and a request for public comment, is expected later this summer.

Pig Tales Scotland's Roslin Institute, a pioneer in cloning sheep, made headlines again this week when reporters learned that it was halting its efforts to engineer pigs that could grow spare organs for transplant into humans. According to numerous press reports, Geron, the California-based company that financed the xenotransplant research, was pulling the plug over concerns that the pig organs might transfer dangerous viruses to people.

It just isn't so, company and institute officials said on 14 August. Disease concerns "were not the basis for the decision to refocus the funding" to other cell biology and genetic engineering projects, Roslin chief Grahame Bulfield said in a statement. "Our decision to allocate funding reflects current strategic priorities," which include harnessing Roslin's cloning expertise to Geron's work with stem cells, added Geron's David Greenwood.

The decision makes sense, industry watchers say, as other companies appear to be in a better position to profit from any xenotransplant breakthroughs. Says one: "Geron is playing to its strengths."

he says, the blast can spread "like a fire through a field of dry grass." The fungus has a harder time finding a compatible host in a mixed environment.

Martin Wolfe, a plant pathologist and research director of the Elm Farm Research Center, an organic farming research center in Hamstead Marshall, Newbury, U.K., supports the approach but notes that the mixture must be tailored to local growing conditions. "This is a useful tool," says Wolfe, who has written a commentary in the same issue. "But you can't just rush in and plant together anything you like."

The message from Zhu's study appears to be spreading through Yunnan Province, where this year 40,000 hectares were planted in the mixed pattern, he says. The payoff, he adds, is easy to measure for farmers: "more rice and more money." **–DENNIS NORMILE**

MICROBIOLOGY

A Weak Link in TB Bacterium Is Found

Easily the most successful human pathogen in the world, the bacterium that causes tuberculosis infects one-third of the world's population. Often acting in deadly combination with AIDS, TB kills 2 million to 3 million people per year, more than any other infectious disease. The secret of the pathogen's success is that it can linger undetected in the lungs for decades, hiding from the macrophages that aim to chew it up and spit it out. Now a team of researchers has uncovered a vulnerability in this resilient bug that suggests new ways to starve it out of its bolt-hole.

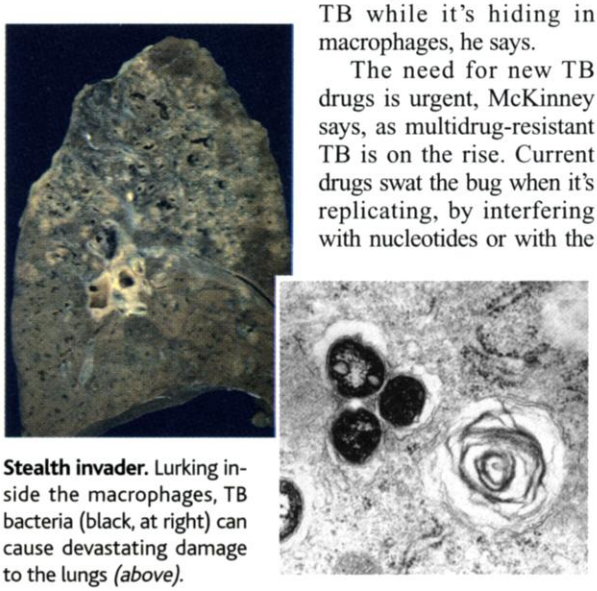
When *Mycobacterium tuberculosis* infects a person for the first time, it proliferates for a few weeks until the immune system marshals its defenses. The two then reach a stalemate, says John McKinney of The Rockefeller University in New York City, part of a four-institution team reporting its findings in the 17 August issue of *Nature*. This persistent state—the pathogen population doesn't increase, but the immune system can't get rid of the bacteria already ensconced—can last a lifetime, with the person suffering no obvious ill effects. But in 10% of those infected, TB will erupt into full-blown disease in response to various stresses or if the immune system is compromised.

During its latent days inside macrophages, the bacterium is stuck with a restricted diet: It eats carbon from lipids via a pathway called the glyoxylate shunt present in bacteria and plants. The TB bacterium also builds amino acids via the oft-memorized Krebs cycle, explains McKinney, but "we went after the glyoxylate shunt because it's the only [pathway the bacteria use for metabolism] not found in humans." Working with William Jacobs Jr. at the Albert Einstein College of Medicine in the Bronx, he created a knockout *M. tuberculosis* that lacks an enzyme called isocitrate lyase (ICL) that is critical for this pathway. Study collaborator David Russell of Cornell University in Ithaca, New York, discovered that ICL levels are elevated in *M. tuberculosis* when it's in its latent phase. Normal TB bacteria burrow into macrophages in mice and make themselves at home indefinitely, but McKinney's altered bacteria that can't produce ICL were wiped out by the animals' immune system.

"One of the things we don't understand is how *M. tuberculosis* can sit around in tissue for years or decades," says Jo Colston, an expert on microbial pathogenesis at the National Institute for Medical Research in London who was not involved in the study. "Obviously, if you can hit a protein that enables [the bacterium] to survive, that represents a potential therapy target."

McKinney and colleagues are searching for such compounds. In a second publication in the August issue of *Nature Structural Biology*, they describe the protein structure of ICL. They also identify two compounds that smother the active end of ICL and shut down the enzyme, thus preventing it from playing its part in the glyoxylate shunt. X-ray crystallographer James Sacchettini of Texas A&M University in College Station, a collaborator on both publications, says his group, working with research sponsor Glaxo Wellcome in the United Kingdom, will screen hundreds of thousands of additional compounds. Those that stymie ICL have potential to serve as drugs that can starve TB while it's hiding in macrophages, he says.

The need for new TB drugs is urgent, McKinney says, as multidrug-resistant TB is on the rise. Current drugs swat the bug when it's replicating, by interfering with nucleotides or with the

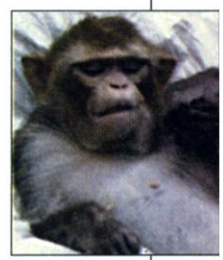


Stealth invader. Lurking inside the macrophages, TB bacteria (black, at right) can cause devastating damage to the lungs *(above)*.

CREDITS: (LEFT TO RIGHT) IMAGE FROM WEBPATH, COURTESY OF EDWARD C. KLATT; DAVID G. RUSSELL/CORNELL UNIVERSITY

ScienceScope

Defining Distress Plans by the U.S. government to change the way researchers characterize pain and distress in lab animals is drawing reaction from biomedical and animal-rights groups. In July, the U.S. Department of Agriculture (USDA) asked for comments on the new guidelines, which are supposed to help researchers spot and lessen discomfort in lab animals. Among other things, the plan defines "distress" as stress that has "negative effects on [an animal's] well being."



Last week, the Federation of American Societies for Experimental Biology (FASEB) said it would prefer a different definition, adopted by the National Research Council in 1992. It describes stress as "an aversive state in which an animal ... shows maladaptive behaviors." FASEB also wants practical rules that rely on the "professional judgement" of researchers and veterinarians.

The Humane Society of the United States and other groups, however, want USDA to adopt a Canadian-style scheme that ranks pain and distress into several categories, based on common lab procedures. "We need a scale with very clear-cut markers," says John McArdle, director of the Alternatives Research & Development Foundation of Eden Prairie, Minnesota. Other ideas may still surface, as USDA will receive comments until at least 8 September.

Orange Alliance At the urging of Senator Tom Daschle (D–SD), the National Institute of Environmental Health Sciences (NIEHS) is trying to team up with Vietnamese scientists to conduct studies of the health and environmental effects of dioxin. The chemical, implicated as a cause of cancer and other disorders, was present in the defoliant Agent Orange, which U.S. forces sprayed widely during the Vietnam war. Today, some Vietnamese carry tissue concentrations of dioxin that are up to 20 times higher than those found in people living in the United States.

This week, NIEHS gathered a group of epidemiologists and toxicologists in Monterey, California, to discuss research strategy and the resources needed to perform epidemiological studies in Vietnam. Later this year, NIEHS scientists plan to meet with their Vietnamese counterparts, with joint studies set to begin in 2002. "That is, assuming the Vietnamese are interested," says NIEHS's Chris Portier.

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