CANCER RESEARCH

DNA Repair Defect Tied to Mutated TGF-β Receptor Gene

When your car breaks down because an incompetent auto mechanic didn't maintain it properly, you may know whom to blame, but you won't necessarily know exactly what went wrong. Was it the clutch? The alternator? The transmission?

In the same way, when researchers found that mutations in genes needed for a type of DNA repair called mismatch repair—one of the cell's mechanics—cause an inherited form of colon cancer known as hereditary nonpolyposis colorectal cancer (HNPCC), they didn't know exactly how the resulting botched repairs lead to cancer. They assumed that the fault lay with damaged genes that trigger tumor development when the cellular mechanic failed to fix them, but they had no clue as to what those genes are. And the genes' identity was of great interest, because tumors with mismatch repair defects, which include sporadic (nonhereditary) cancers as well as those due to HNPCC, are common, accounting for some 15% of colon cancer cases.

But now, one of the broken genes has been found—and it's in the brake system. On page 1336, a team including Sanford Markowitz and James Willson of University Hospitals of Cleveland and Case Western Reserve University, Michael Brattain of the Medical College of Ohio in Toledo, and Bert Vogelstein, a Howard Hughes Medical Institute investigator at Johns Hopkins University School of Medicine, report that in colon cancer cells with a particular kind of genetic instability caused by mismatch repair, mutations accumulate in a specific gene: the gene for one of the receptor proteins through which a protein called transforming growth factor–β (TGF-β) exerts its effects. Those mutations, they find, inactivate the gene. "It wasn't known what the key [mutation] targets were," says cancer gene expert John Minna of the University of Texas Health Science Center in Dallas. "Now it's clear that at least one is this TGF-β receptor."

The new finding provides a plausible mechanism for how defective DNA repair contributes to cancer development. Despite being called a "growth factor," TGF-β actually inhibits the growth of epithelial cells, the type of cell from which colon cancers originate. Loss of the receptor protein should therefore render cells unresponsive to TGF-β's growth-inhibitory effects, removing one of the brakes that normally hinder progress toward malignancy. "I think [the paper] is very exciting. It underscores the key role of TGF-β in carcinogenesis," says Anita Roberts of the National Cancer Institute (NCI), who has long studied TGF-β's role in cancer.

Adding to the excitement over the theoretical implications of the work are the potential clinical applications: Screening for the mutant TGF-β receptor gene may aid in diagnosing colon cancer and two other cancers common in HNPCC families, ovarian and endometrial cancer. Ultimately it may even be possible to treat cancers in which the TGF-β receptor gene is defective by using gene therapy to transplant functional copies of the gene into patients' cells.

The discovery that the receptor gene is mutated in some of the colon cancer cells may also help solve an additional cancer research mystery. Several groups, including Markowitz's, have found that colon and other types of cancer cells often stop responding to TGF-β as they become more malignant. Researchers didn't know how that happened, but it now appears that in some cancers, the receptor gene mutations are to blame.

Markowitz, Willson, and Brattain originally set out to try to explain the mystery of the loss of response to TGF-β. The receptor, which is composed of two proteins, RI and RII, was an obvious place to look for the answer, as it must be present and functioning for TGF-β to exert its normal braking effect. And other groups had already found indications of problems in the receptor: It proved to be absent from the membranes of tumor cells from a human lymphoma patient, for example, and other tumor cells turned up deficient in messenger RNAs (mRNAs) made by the receptor genes. Because production of mRNAs is the first step in protein synthesis, that suggested that the genes might not be working properly.

Following up on these observations, Markowitz, Brattain, Willson, and their colleagues screened 38 colon cancer cell lines for the mRNAs corresponding to the two receptor genes. They found that all the cell lines made normal amounts of the RI mRNA, but 12 of them had little or no mRNA for the RII gene. And that's where Markowitz made what Vogelstein, whose group was one of those that originally found that DNA repair is defective in patients with HNPCC (*Science*, 4 November 1994, p. 728), calls an "astute observation."

The observation was to note a close correlation between the tumor cell lines with low or absent RII mRNA levels and those having a genetic abnormality called "microsatellite instability." This abnormality, in which the microsatellites (DNA segments consisting of a few bases repeated over and over) are either longer or shorter than they should be, is a common result of defective mismatch repair. This repair pathway fixes the type of damage that occurs when DNA is being copied and one strand slips relative to the other, and repeated sequences are particularly prone to such slippage. But mistakes may occur in regular genes as well, and thus the correlation immediately suggested "that the receptor gene was being inactivated by mutation in these cells," Markowitz says.

Subsequently, Lois Myeroff of the Markowitz group and Ramon Parsons of Vogelstein's lab confirmed that was indeed the case by showing that the RII genes in the cells lacking the corresponding mRNA have inactivating mutations located within a sequence containing 10 adenine bases in a row—what Markowitz calls "a mini-microsatellite." This may explain, he says, why the gene is such a tempting mutation target: "There is a design flaw sitting right there in the middle of the gene that makes it uniquely susceptible to being killed." And the mutations are not just an artifact of cells in culture. The researchers had original tumor tissue from which four of

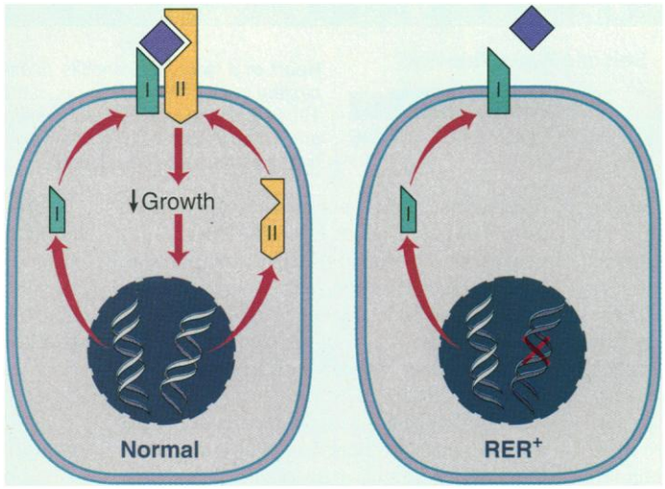


ILLUSTRATION: K. SUTLIFF

Wiped out. In cells making the RI and RII receptor proteins, TGF-β *(purple diamond)* inhibits cell growth. But in RER+ cells, with the DNA repair defect, both copies of the RII gene are mutated, releasing the cells from TGF-β's growth-inhibitory effects.

the cell lines were derived, and these, too, had the mutations, although normal cells from the same patients did not. And since the paper was written, Vogelstein says, the researchers have screened dozens of primary tumors from HNPCC tumors, and "nearly all have the same mutations."

Even that impressive evidence doesn't necessarily prove that the mutation contributes to the cancer development. "You have to be careful about ascribing a role [to a mutation], since so many genes are mutated in cancer cells," Vogelstein cautions. But the researchers have evidence that the mutation in the RII gene is more than just an innocent bystander. When the Brattain-Markowitz-Willson team transferred a good copy of the gene into a line of colon cancer cells that lack it and normally form fast-growing tumors when injected into nude mice, they found that the cells lost their tumor-forming capacity. "This demonstrates that there really is a biological consequence of the mutation," Brattain says.

Researchers who study TGF-β are thrilled by these results. NCI's Roberts notes that while there was ample reason to suspect that a receptor defect might be involved in tumor cells' loss of responsiveness to TGF-β, "this really nails it down" for a human cancer. Another TGF-β pioneer, Harold Moses of Vanderbilt University School of Medicine in Nashville, Tennessee, agrees: "This is the first demonstration of a mechanism for the loss of the growth inhibitory response, and to tie it in with the repair defects is particularly interesting."

In addition to providing a possible solution to the problem of what causes loss of response to TGF-β, these results suggest, says Minna, that the RII gene may be a critical kind of gene called a tumor suppressor, whose loss or inactivation may lead to cancer. He points out that the gene maps to an area on chromosome 3 that is thought to contain a tumor suppressor because it is deleted in several cancers. His own team, he says, is exploring whether loss or inactivation of the gene might contribute to development of small-cell lung cancer. Indeed, he says, "several components within the pathway [by which TGF-β inhibits growth] could be tumor suppressors."

On the clinical front, Markowitz and Willson want to explore whether it is possible to detect HNPCC colon and ovarian cancers by screening for cells that carry the RII mutation in blood or stool. And beyond that lies the goal of using gene replacement therapy to treat cancers that have lost their responsiveness to TGF-β by putting in a good copy of the RII gene. That's a long way off, but the new work at least opens up the possibility of doing what the cell's mechanic should have been doing all the time.

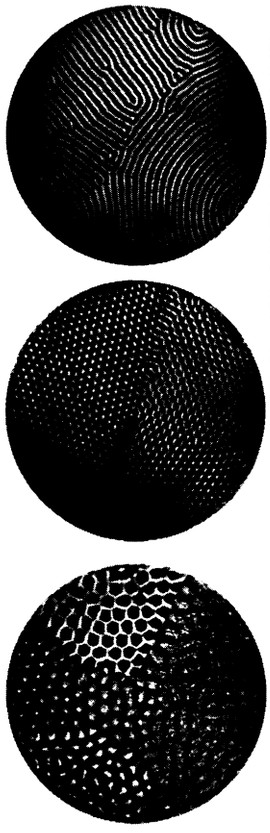
–Jean Marx

PHYSICS

Grown-Up Physicists Play Serious Games in the Sandbox

An old adage holds that all problems in physics are either trivial or insoluble. Those categories can actually be near neighbors. Take the bouncing, flowing, mixing, oscillating, avalanching substances called granular materials—of which sand is the most familiar. "Sand," says University of Chicago physicist Heinrich Jaeger, "we associate with something terribly simple. Something whose behavior we can obviously predict. But if we look more closely," says Jaeger, who recently helped organize a conference on granular materials,* "we are faced with an unbelievable complexity of outcomes."

That mix of simplicity and complexity won't come as a surprise to industrial engineers, who have struggled for decades with fertilizers that plug hoppers and pharmaceuticals that won't stay mixed as they form pills, to cite just two examples. Granular materials are polymorphous: They can resemble solids, liquids, or gases, depending on the situation, and engineers have learned the hard way that a weird amalgam of known and unknown physical laws governs their behavior. But physicists have been latecomers to the sandbox. "A few years ago you used to be a laughingstock if you were working on sand," says Anita Mehta of the University of Birmingham in the United Kingdom.



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All shook up. A layer of particles shifts from one surface pattern to another as the vibration amplitude changes.

Now, she says, "it has become rather trendy." The complex behavior of sand and other granules has enchanted physicists by giving them a model of other difficult areas of physics. "The sandpile is a metaphor for a lot of systems in physics that we have been struggling with for a long time," says Jaeger. As speakers at the conference showed, granular materials can mimic the roiling convection cells that form in fluids, undergo "phase transitions" analogous to those of solids changing from one crystal structure to another, and form bizarre, fingerlike clumps through processes that may mimic structure formation in the early universe. And these phenomena are yielding new insights for the industrial engineers who have traditionally struggled with granular media on their own.

Like an avalanche triggered by a single pebble tumbling down a slope, much of the recent surge of interest in granular materials began with the 1987 publication of a paper by Per Bak, Chao Tang, and Kurt Wiesenfeld, then at Brookhaven National Laboratory. The paper laid out a notion these researchers dubbed "self-organized criticality." They proposed that under the pressure of outside stimuli, a system of many complicated, interacting parts—anything from a sandpile to the stock market—will organize into a precarious state, far from stability, that is prone to unpredictable fluctuations. In the case of a sandpile, the theory predicts that as sand is added to the pile, it will slough off not in regular, catastrophic avalanches but in an unpredictable series of small and large ones.

Whether nature really behaves this way is still a matter of debate. But by getting physicists to take a serious look at sand, the proposal opened up the field. At the University of Chicago, for example, a group that includes Jaeger, Sidney Nagel, and Chu-Heng Liu (now at the Exxon Research and Engineering Company in Annandale, New Jersey) started by looking for self-organized criticality in sandpiles. They failed to see it, says Jaeger, "but got intrigued by all of the other things that happen in sandpiles" and other granular aggregations. Among them was the "Brazil nut phenomenon"—the puzzling tendency of the largest granules in a container to rise to the top when it is shaken.

Two years ago Jaeger and Nagel, along with Chicago colleagues James Knight and Edward Ehrichs, studied the phenomenon by filling a cylindrical container with glass beads, some of them dyed black to serve as

* Dynamics of Granular Materials: Understanding and Control, May 11–13 at the University of Chicago. Organizers: Heinrich Jaeger, Elizabeth Grossman, Sidney Nagel, and Yunson Du.