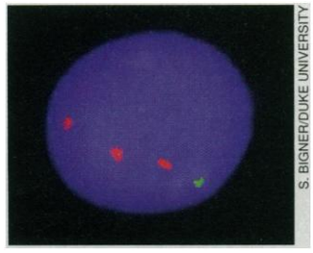
New Tumor Suppressor Found—Twice

Two research teams have separately homed in on a tumor suppressor gene, the loss or inactivation of which may be important for the progression of brain, prostate, and other cancers

When Jing Li joined Ramon Parsons at Columbia University's College of Physicians and Surgeons last year to hunt for breast cancer genes, he expected the work to be intense. But when news reached the lab that another team might have cloned the same gene his lab was working on—a tumor suppressor, the inactivation of which seemed to contribute to the development of both prostate cancers and the highly malignant brain tumors known as gliomas—Li got a true taste of just how high the stakes have become as academic and corporate labs scramble to find important disease genes (see sidebar). Now, the race for this gene has ended in a dead heat.



S. BIGNER/DUKE UNIVERSITY

Lost gene. Brain-tumor cells often lack a copy of chromosome 10 (green) and gain a chromosome 7 (red).

On page 1943, Li, Parsons, and their colleagues report that they have cloned the tumor suppressor, which resides on chromosome 10. And in the April issue of *Nature Genetics*, cell biologist Peter Steck of M. D. Anderson Cancer Center in Houston and Sean Tavtigian of the biotech firm Myriad Genetics in Salt Lake City will announce that they have found the same gene.

Called *PTEN* (for *phosphatase and tensin homolog deleted on chromosome 10*) by the Parsons group and *MMAC1* (for *mutated in multiple advanced cancers 1*) by Steck and his colleagues, the new gene joins some 16 other known tumor suppressors. But while it's far from the first such gene discovered, cancer researchers are enthusiastic, because the early data indicate that *PTEN* might rank in importance with *p53*, *retinoblastoma*, and *p16*, tumor suppressors that have been linked to several types of tumors. "[*PTEN*] seems to be a major gene in some pretty important cancers," says Kenneth Kinzler, a molecular geneticist at Johns Hopkins University. In addition to prostate cancer, which afflicts some 317,000 men every year in the United States, and gliomas, which strike another 15,000 people, these might include breast and kidney cancer.

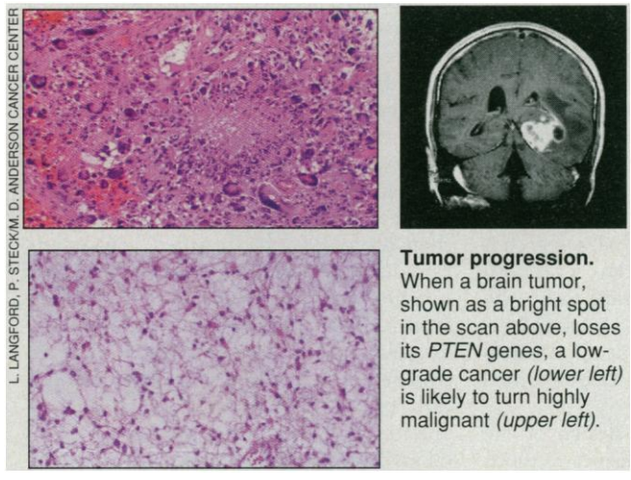
But equally intriguing, says molecular biologist Stephen Friend of the Fred Hutchinson Cancer Research Center in Seattle, is the apparent mode of action of the PTEN protein. Its amino acid sequence indicates that it resembles two different types of proteins: tyrosine phosphatases, which are enzymes that remove phosphate groups from the amino acid tyrosine in other proteins, and tensin, a protein that helps connect the cell's internal skeleton of protein filaments to its external environment.

Cancer researchers suspected that tyrosine phosphatases might be tumor suppressors because they directly counter the actions of another set of enzymes, the tyrosine kinases, which add phosphates to tyrosines and are part of the cell's growth-stimulating pathways. But there had been no direct evidence for that—until now. "This is proof of a long-held speculation that phosphatases would be important," Friend says. In addition, the tensin resemblance suggests that PTEN might help cells stay in their normal locations within a tissue. Its loss, then, might be one of the steps that give tumor cells the ability to spread.

Parsons began the current work about a year ago, when he joined forces with Michael Wigler of Cold Spring Harbor Laboratory on Long Island to apply a technique Wigler had developed earlier to the hunt for breast cancer genes. Called representational difference analysis (RDA), the technique can identify abnormalities in DNA by comparing the equivalent sections of DNA from normal and diseased cells (*Science*, 12 February 1993, p. 946). By 1996, the technique had already helped researchers home in on *BRCA2*, the second of two genes that cause hereditary susceptibilities to breast cancer.

Many of the gene changes that lead to cancer are not inherited, however, but simply develop in specific cells, like those in the breast epithelia. To find such noninherited gene changes, Wigler had applied his method to cells from 12 primary breast tumors, identifying about a dozen possibilities for such cancer-causing gene changes, including a deletion on chromosome 10. Parsons was particularly interested in following up on that observation. Chromosome 10 is completely or partially missing in a variety of cancers, especially the aggressive brain tumors called gliomas—a prime indication that it carries a tumor suppressor. Researchers also suspected that it carries the gene responsible for a rare inherited disorder called Cowden disease, whose victims are predisposed to breast and other tumors.

To narrow down the location of the suspected tumor suppressor, Wigler and the Parsons team examined cells from 65 human breast cancers to see whether their DNA lacked any of nine genetic markers located in the part of the chromosome that the RDA had identified as abnormal. One marker was absent in two of those samples, and when it also proved to be missing in some prostate and glioblastoma cell lines, Parsons and Wigler knew they were closing in on the gene. By October 1996, they were ready to try a technique called exon trapping to pull it out. This involves looking for messenger RNAs made by the deleted region, then using them to find the corresponding exons, which are the protein-coding regions of a gene.



L. LANGFORD, P. STECK/M. D. ANDERSON CANCER CENTER

Tumor progression. When a brain tumor, shown as a bright spot in the scan above, loses its *PTEN* genes, a low-grade cancer *(lower left)* is likely to turn highly malignant *(upper left)*.

They found two exons. To get the rest of the gene, the group consulted the GenBank database, which includes not only the sequences of full genes but also the short DNA pieces called expressed sequence tags (ESTs). More than a dozen ESTs in the database matched different parts of the exons. Aided by a computer program called UNIGENE, which groups ESTs that seem to be part of the same gene, the researchers were

Prepaper Publicity Ignites Race to Publish

In mid-January, Ramon Parsons received a phone call that is every researcher's worst fear. Just weeks earlier, the molecular biologist, who works at Columbia University's College of Physicians and Surgeons, and his research associate Jing Li had finally nailed the tumor-suppressor they had been hunting for the past year. It was potentially a major prize, but they still had to verify that the gene was indeed a tumor suppressor—one whose loss or inactivation can lead to cancer development—and determine the range of tumors with which it might be involved.

But just as they were anticipating the fruits of success, one of their collaborators, molecular biologist Michael Wigler of Cold Spring Harbor Laboratory on Long Island, called to say that he had just read in a biotechnology newsletter that Myriad Genetics had also found a tumor-suppressor. There was scant information in the press release, but what was there set off alarms. Myriad had linked its gene to malignant brain tumors called gliomas—just as Parsons had. The two genes, he feared, were the same.

What happened over the next few weeks, as both the Parsons and Myriad groups rushed to get papers in press and file patents on the gene, is testimony to how complex life has become for researchers tracking down disease genes. With industrial collaborations on the rise, the competition has grown more intense, and patenting and stock-market worries are having an ever greater influence on how scientists go about their business.

The immediate cause of Parsons's panic was a press release that Myriad put out on 22 January. This release simply highlighted the gene's role in gliomas, without mentioning its chromosomal location, or giving any information about its protein product or other cancers the gene might be involved in. Also missing was any indication that the work had been published, or was at least submitted for publication. "I thought it was bizarre, because they were announcing a discovery without publishing it," Parsons recalls.

Mark Skolnick, Myriad's vice president of research, says the company put out the release to guard against possible charges of insider trading by the U.S. Securities and Exchange Commission. Sean Tavtigian of Myriad notes that the company was about to enter one of the quarterly periods during which employees with stock options are allowed to trade their Myriad stock, and wanted to make sure that the public knew what the employees knew—that it had the glioma gene in hand—during that period. "We have to be uniform in our release of information," says Skolnick. "There's a potential liability if information gets out in an uneven fashion."

But when the release was mentioned in the biotech newsletter, *Bioworld*, it also alerted Parsons to the competition at Myriad. "From reading the press release, [it seemed] we were farther along than they were," says Parsons. Nevertheless, he worried that if the two groups had converged on the same gene, this announcement might jeopardize his chance to get credit for the discovery. "Do you know how hard it is to publish in a small lab if you're second?" Parsons asks.

Li and two graduate students worked around the clock for the next 4 days screening various tumor samples, mostly primary brain tumors, to verify that the gene is indeed missing or aberrant, as would be expected for a tumor suppressor (see main text). But they skipped some of the tests they had planned to show that the gene is aberrant in more kinds of tumors, and also put off filing a patent on the gene until the paper was submitted. "My interest was to get a paper out the door," Parson says. Indeed, on 31 January, as soon as the paper was finished, Li flew to Washington, D.C., to hand-deliver it to *Science*. "It was pretty crazy," he says.

Meanwhile, Myriad's academic collaborator on the project, Peter Steck of the M. D. Anderson Cancer Center in Houston, found himself caught up in Myriad's commercial priorities. "The first emphasis was patenting," he explains. In addition, he was racing to meet a renewal deadline for his grant. He didn't get to his paper until later, even though he began hearing through the "industrial grapevine," as Steck calls it, that they had competition. The paper was submitted in late February to *Nature Genetics*, accepted within a week, and published 3 weeks later, technically 4 days after Parsons's report.

But while Parsons beat Steck and Myriad to publication, albeit by a narrow margin, there's no telling yet which group will wind up with the patent. And perhaps neither will. Both groups' searches led them to the GenBank computer database of gene sequences, which already turned out to contain several small DNA bits, called expressed sequence tags (ESTs), that fell inside the gene. A computer program had even grouped those ESTs into a tentative gene, which contained a sequence indicating that its protein product is a dephosphorylating enzyme. Myriad's Tavtigian points out that this could mean that a company that has generated many ESTs—Human Genome Sciences in Rockville, Maryland—may have beaten both Steck and Parsons to the Patent Office. That company declined comment on that possibility. –E.P.

then able to piece together the whole gene, using the ESTs as guides for sequencing it.

In contrast to Parsons's 1-year blitz for the chromosome 10 gene, Steck's progress has been slow and steady, and he began his quest in gliomas rather than breast cancers. To try to find the crucial chromosome 10 gene that is missing in many of these brain cancers, Steck and his colleagues began adding progressively smaller pieces of the chromosome back to cultured glioma cells. The idea was to demonstrate that one or more genes on the chromosome could reverse some of the cancerous changes in the cells, and then to narrow the search for those genes to ever smaller pieces of the chromosome.

This approach got the researchers to within 5 million bases of the gene. To close in further, they determined whether glioma samples lacked a genetic marker located within that region, and by last summer had found four samples in which both copies of chromosome 10 were missing that marker. There was a 75,000–base pair overlap in the missing DNA in these samples—a gap that presumably extended over their tumor suppressor.

However, the researchers still had a lot of DNA to sort through, and Steck thought it might be too big a project for his three-person lab group. He then went for help to Myriad, a company experienced in locating and sequencing genes, having done so for both *BRCA1* and *-2*, and *p16*. In November, Myriad's Sean Tavtigian stepped in; with Steck, he completed the hunt for the gene—all in about a month, Tavtigian says, using basically the same approach as the Parsons group. They also found signs that the gene is involved in some kidney, breast, and prostate cancers, as well as in gliomas.

Although this team called the gene *MMAC1*, its sequence shows that it is the same as *PTEN*. "We started from two different places for two different reasons and got to the same place at the same time," says Steck, who was unaware of the Parsons effort until a few months ago. "We confirm each other's work."

Both groups also attest to the importance of the gene. The Parsons group, for example, confirmed the Steck group's evidence that the gene is missing in many gliomas, as well as in some breast cancers. Their results hint that the gene is also important for prostate cancer. It was missing or altered, for example, in all four

samples of the cancer that the Parsons group studied. Indeed, Johns Hopkins's Kinzler says, "there have been other candidate [prostate cancer] genes proposed, but I think this is the real McCoy." And he predicts, "the chances are, it's going to be involved in other cancers."

Researchers still have a lot to do to find out just how the gene's loss could contribute to these cancers, although its sequence provides some important clues. As a phosphatase, the PTEN protein may counteract the work of the growth-stimulating kinases, which can help make cells cancerous when they are mutated into an overactive form. The researchers have not yet shown directly that the protein is a phosphatase, however, nor have they identified any possible targets for its phosphate-removing activity.

The cytoskeletal connection might also help explain the abnormal growth of cancer cells. Because of its links to the protein matrix outside the cell, the cytoskeleton is thought to be part of the system that helps cells know that they are in contact with neighboring cells. Normal cells tend to stop multiplying when they encounter their neighbors, but cancer cells often keep dividing, as if they never got the message to stop. PTEN's absence might be what blocks the message. PTEN may also somehow help anchor cells, in which case its loss may enable a cell to metastasize. "If [*PTEN*] does have a role in cell motility or cell structure, that might be quite interesting," says Eric Fearon, a cancer geneticist at the University of Michigan, Ann Arbor. How the protein's proposed roles as a phosphatase and a cytoskeletal protein might relate to each other is unclear, however.

Even before researchers know how the gene works, it may prove useful to clinicians. Tavtigian points out that if this gene is the one mutated in Cowden disease, it could form the basis of a prenatal diagnostic test. And if the loss of the gene helps a cancer invade other tissues, then *PTEN*'s status may help oncologists predict how malignant a glioma or prostate tumor will be—information that could help clinicians decide how aggressive they should be with surgery, chemotherapy, or other treatments. "If you had a molecular marker that could aid a clinician in that decision, that would be very significant," Steck suggests.

And then there's the possibility that the PTEN work might provide guides to better cancer therapies by leading researchers to protein it normally dephosphorylates, putting the brakes on cell growth. A drug that either blocks the phosphorylation of the protein or removes phosphates from it might cure a cell of any cancerous tendencies.

Given all this potential, Li's life will not likely slow down any time soon, Parsons notes: "I think it's going to continue to be crazy here for at least another 6 months."

–Elizabeth Pennisi

MATERIALS SCIENCE

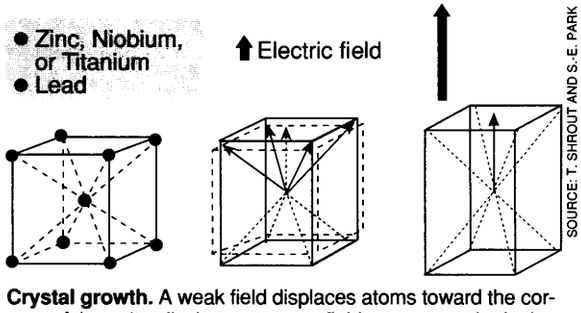
Shape-Changing Crystals Get Shiftier

A talented family of materials has gained some even more gifted members. So-called piezoelectric crystals have the unique ability to swell or shrink when zapped with electricity, as well as give off a jolt of juice themselves when compressed or pulled apart. Engineers have exploited this trait for decades to convert mechanical energy to electricity and back again in applications ranging from phonograph needles to telephone speakers.

Now, a pair of researchers from Pennsylvania State University has bred new piezoelectric wunderkinds, some of which display an effect 10 times greater than that of current family members. A paper by the researchers, materials scientists Thomas Shrout and Seung-Eek Park, is scheduled to appear this spring in the inaugural issue of the journal *Materials Research Innovations*, but early word of the new work is already turning a few heads. "It's an exciting breakthrough," says Eric Cross, another piezoelectric materials expert at Penn State, who is not affiliated with the project. "Improvements by a factor of 10 are not easy to come by in a field that's 50 years old and considered mature." If the materials are commercialized, as Cross and others believe they will be, they could usher in a new generation of piezoelectric devices that would improve everything from the resolution of ultrasound machines to the range of sonar listening devices.

Piezoelectric materials owe their abilities largely to the asymmetrical arrangement of positively and negatively charged atoms in their crystal structure. The positive and negative charges balance out in each of the crystal's unit cells—its basic repeating units—but the positive charges, for instance, may be weighted toward the top of each cell. An electric field can displace the charges even farther, which distorts the overall shape of the unit cell and of the crystal as a whole. The process can also run in reverse: Squeezing or stretching the material shifts the charges relative to each other, redistributing electric charge around the surface of the crystal, which can produce a small electric current.

The usual showcase for these properties is a cheap ceramic material called PZT, containing millions of crystalline grains in different orientations. PZT, which is composed primarily of lead, zirconium, titanium, and oxygen, can deform by as much as 0.17% in a strong applied field. To boost this shape-shifting ability, researchers have tried to grow single crystals of PZT, in which all the unit cells would line up in the same direction. Their contributions to the piezoelectric effect would also line up, enhancing it. But because PZT's components tend to separate during processing, the ceramic is extremely difficult to grow as a single crystal, says Shrout.



Crystal growth. A weak field displaces atoms toward the corners of the unit cells, but a stronger field rearranges the lattice.

To coax the material into forming single crystals, Shrout and Park tried varying its composition. They settled on a couple of different mixtures, such as a combination of lead, zinc, and niobium spiked with varying amounts of lead-titanate (PT). The researchers found that a small admixture of PT—less than 9%—yielded materials that not only grew into single crystals, but also ended up with piezoelectric abilities that are enhanced more than they expected.

Just why that is, "we still don't know for sure," says Shrout. But he and Park believe that at least part of the enhancement is due to the fact that an electric field applied to the new materials does more than just shift a few atoms around in the unit cell, as in PZT: "We think it causes the whole crystalline lattice structure to change from one form to another," says Shrout. The changed crystal structure, in turn, frees individual atoms to respond more strongly to the field, increasing the overall distortion of the material. Likewise, a mechanical distortion probably produces a similar lattice shift, enabling the material to generate more current than standard PZT.

Whatever the reason for the effect, it's likely to be very useful, says Robert Newnham, another piezoelectricity expert at Penn State. The new crystals will undoubtedly cost more than ceramics like PZT, says Park, because growing single crystals is a slow and painstaking process. But he adds that he and Shrout are working on ways to speed it up. If they succeed, the new piezoelectric wunderkinds could grow up to live expansive lives indeed.

–Robert F. Service