MOLECULAR IMAGING

New Probes Open Windows on Gene Expression, and More

A few days before Christmas 1895, Wilhelm Röntgen snapped a couple of spooky-looking pictures that changed the world of medicine. The images, taken with newly discovered x-rays, revealed the bones of his wife's hands as a set of shadowlike features. Medical imaging has been on fast forward ever since. In recent decades, researchers have added numerous imaging tools, such as positron emission tomography (PET) and magnetic resonance imaging (MRI), that offer more detailed insights into the inner workings of the body. Yet, for all the prowess of these techniques, they show fundamentally the same kind of thing as Röntgen's x-rays: general anatomical features, such as organs, tissue masses, and metabolically active tissues in the brain. Useful as they are, such images can't answer crucial questions, such as whether a tumor is malignant or benign.

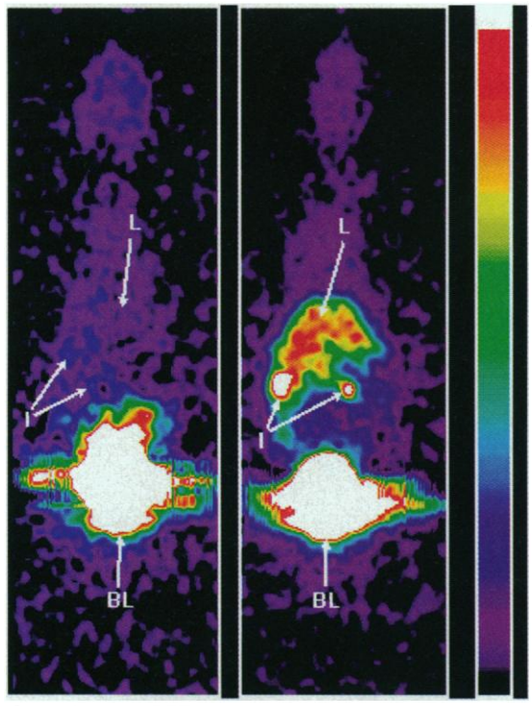
Now researchers around the world are furiously competing to launch a new age in medical imaging that looks beyond general anatomy into the molecular workings of tissues. By developing clever probes that give off a detectable signal when they encounter a specific molecule, such as the product of a particular gene, researchers hope to pin down a tissue's exact metabolic state. Investigators have already used this strategy to track the transfer of genes in gene-therapy experiments and map the distribution of an animal's own proteins. Down the road, they hope to build on these successes to perform a variety of feats, from imaging the effectiveness of cancer therapy to mapping when different genes get turned on during development—all without removing tissue with a scalpel and testing it in the lab.

"This really marks a new paradigm shift that's taking imaging to the next level," says Michael Phelps, a PET imaging expert at the University of California, Los Angeles (UCLA), who is developing novel molecular probes to track gene therapy and cancer treatment. "We are at the edge of a revolution," adds Elias Zerhouni, a radiologist and biomedical engineer at Johns Hopkins University in Baltimore.

This revolution is being fomented by advances such as the unmasking of genes involved in cancer and other diseases and the exact shape of the proteins for which they code. That knowledge, in turn, is allowing chemists to tailor specific new molecular imaging probes that can put one gene or protein in the spotlight while all else remains dark. To turn on the spotlight, this revolution relies on tried and true imaging techniques such as PET, which tracks gamma rays from tiny amounts of radioactive elements injected into the body.

In the standard PET technique, researchers add a radioactive tag to glucose, which brain cells take up when they are metabolically active. A PET scan can then trace which parts of the brain are busy during specific tasks, such as reading or listening to music. In a newer PET variant, neuroscientists add the radiolabels to organic compounds that bind selectively to particular types of receptors that decorate the outside of nerve cells in the brain. Using this approach, researchers have mapped the distribution of nerve cells that use dopamine and serotonin.

Now they hope to reproduce this level of selectivity throughout the body. In 1995, for example, Ronald Blasberg, Juri Tjuvajev, and their colleagues at Memorial Sloan-Kettering Cancer Center in New York City used a laboratory relative of PET—a radioactive scanning technique known as autoradiography—to track the success of a gene-transfer experiment. The Sloan-Kettering team used conventional gene-therapy techniques to introduce into mouse tumor cells a gene from the herpes simplex virus (HSV) that codes for an enzyme called thymidine kinase (TK). HSV-TK adds phosphates to thymidine—a structural component of DNA—and closely related molecules. The researchers then injected the animals with a thymidine analog called FIAU, tagged with radioactive iodine. FIAU readily enters and exits cells. But when it encounters HSV-TK, the enzyme tacks on phosphates and the molecule gets trapped in the cell, where it accumulates and produces a strong enough gamma ray signal to be detected. When Blasberg's team killed the animals and scanned them with an autoradiography machine, areas that expressed the HSV-TK gene lit up.



S. GAMBHIR *ET AL.*, UCLA

Gene therapy. PET scan shows gene expression in liver (L) of live mouse *(right)* compared with control *(left)*. Both show labeled probe in intestines (I) and bladder (BL).

Autoradiography has a distinct drawback: It doesn't work with live animals, because it can only pick up signals through thin slices of tissue. But the Sloan-Kettering team—as well as another led by Michael Phelps and Sam Gambhir at UCLA School of Medicine—has reported at recent meetings that it has used PET to image the expression of the transplanted gene in live animals. The UCLA researchers, along with another group from Harvard Medical School in Boston, have also developed a slightly different technique in which a transplanted gene induces cells to express molecules on their surface that bind to a radioactive probe.

Phelps, who presented his team's latest work last month at the American Chemical Society meeting in Dallas, says the goal is to use these tracer genes to mark the expression of a therapeutic gene. The idea is to splice the tracer gene into a stretch of DNA alongside the therapeutic gene and a promoter that causes both to be expressed at the same time. Where the tracer gene shows up, "you know you have expression of your therapeutic gene as well," says Phelps. Gene-therapy pioneer Jack Roth of the University of Texas M. D. Anderson Cancer Center in Houston says that type of information is just what the struggling field of gene therapy needs. "We'd love to know what cells the gene is going into and where it's being expressed," says Roth.

Looking to RNA. Researchers are now working on a PET-based technique that would have far broader applications: imaging the expression of native genes. The goal is to develop radioactive tags that would home in on and bind to specific messenger RNA (mRNA) molecules—the chemical signals that turn on cellular production of a protein.

If it works, says Sudhir Agrawal, who heads discovery research at Hybridon, a Cambridge, Massachusetts–based biotech company, the approach "would change the face of diagnostic imaging, allowing doctors to be able to tell if patients are expressing particular genes." By looking for declines in the activity of genes that enable cancer cells to proliferate, says Agrawal, doctors could measure the effectiveness of therapy.

Hybridon and other biotech companies are already working to develop so-called antisense RNA molecules that would bind to mRNAs from such genes, blocking the production of the proteins for which they code (*Science*, 23 May 1997, p. 1192). Phelps and other imaging researchers are hoping to piggyback on this technology, by attaching radioactive labels to the antisense molecules. Progress is slow, but it's beginning to pick up. In the April issue of *Nature Medicine*, a team led by Bertrand Tavitian at the French biomedical agency INSERM in Orsay showed that it could radiolabel antisense molecules and track them through the body.

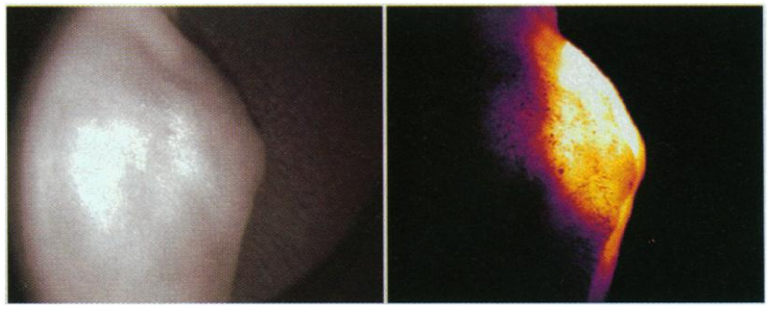
Still, no one has yet shown that they can actually image the antisense once it has bound to its target inside cells, says Phelps. Antisense molecules are quickly broken down by enzymes in the body, and the molecules are charged, which makes it difficult for them to cross cell membranes to find their targets. Both problems keep the antisense RNA from accumulating inside cells to levels high enough to be picked up by an imager. In Dallas, Phelps reported some progress: By doctoring the backbone of their antisense probes, they made the RNA less susceptible to enzymatic degradation. But as for the rest of it, he adds, "we're still not there yet."

Other views. Although imaging mRNA directly remains a work in progress, approaches to mapping the distribution of proteins are further along. Researchers at the California Institute of Technology (Caltech) in Pasadena and at Harvard Medical School, for example, have a scheme that relies on MRI. MRI normally detects the particular magnetic signal from hydrogen atoms, which makes it easy to spot hydrogen-rich water molecules. The technique maps the outline of tissues in the body by looking for differences in water content.

To enable MRI to home in on molecules other than water, the Caltech and Harvard teams took advantage of a standard trick for enhancing the contrast of MRI scans: injecting compounds containing paramagnetic metal ions such as gadolinium(III). These ions contain unpaired electrons that interact with neighboring water molecules to boost their magnetic signal.

The Caltech researchers, led by chemist Tom Meade, surrounded gadolinium with bulky organic groups that prevent it from interacting with water. One of these organic appendages is bound to the gadolinium with a sugar molecule that is vulnerable to the enzyme β-galactosidase. When the enzyme is present, it severs the bond to the sugar, water molecules move in to interact with the gadolinium, and the MRI signal is turned up. In tissues where β-galactosidase is absent, the signal is unchanged.

At a brain imaging meeting held last week at the National Institutes of Health in Bethesda, Maryland, Meade and his colleagues reported that they can map tissues with and without β-galactosidase in living mice. "There's no reason why this can't be applied to detect any enzyme," Meade says, and his team is now developing a set of probes to detect other types of enzymes. They are planning to use their probes to try to track embryonic development in action, to see at just what point different enzymes get turned on in cells.



R. WEISSLEDER, HARVARD

Highlight. Probe fluoresces in near-infrared light when it is cleaved by enzymes in a tumor *(right)*. The same tissue shows few details in visible light *(left)*.

Ralph Weissleder and his colleagues at Harvard and Massachusetts General Hospital in Boston, meanwhile, are developing a less specific technique for boosting the MRI signal. Like some PET scan researchers, Weissleder and his colleagues are turning to genetic engineering for help. At another molecular imaging meeting last February in Bethesda sponsored by the National Cancer Institute (NCI), Weissleder and his colleagues reported that they had coaxed mouse tumor cells to express a modified cell membrane protein that continually pumps paramagnetic iron particles inside cells. Cells expressing this protein lit up in the imager.

Seeing red. At the same NCI meeting, Weissleder reported that he and his colleagues have also made progress in using light instead of radiation or magnetic resonance to detect the signature of specific enzymes associated with tumors. The key is a novel set of probes that fluoresce only when they react with the target enzymes. Weissleder's team starts with a molecule consisting of 10 to 20 chromophores—common organic dye molecules—closely spaced along a backbone of lysine groups. Normally the chromophores fluoresce when hit with infrared light. But in this case they are spaced so tightly that the excitation energy shuttles from one chromophore to another and is eventually emitted as heat instead of light. When the compound is injected into the body, however, it circulates until it encounters enzymes called hydrolases, which snip bonds between lysine groups in the backbone. This sets the chromophores free, allowing them to fluoresce. Because tumors are rich in hydrolases such as cathepsin, Weissleder and his colleagues found that the chromophores selectively light up tumors that had been grafted onto mice.

"It's a very interesting approach that's just in its infancy," says Meade. And because it uses just infrared light, which does not damage cells, it has "enormous potential," he adds. He and others note that because the technique relies on light, it can track enzymes only to a depth of a few centimeters in tissue. But Weissleder points out that the snakelike endoscopes already used by surgeons for minimally invasive surgery should allow the technique to be used throughout the body.

Indeed, each new imaging technique has its own strengths and weaknesses. PET, for example, can pick up the faintest signals, but with only 40 or so PET centers worldwide, it will be hard for doctors and patients to use it clinically. MRI is more widely available and can pick out finer features than PET, but for now it remains less sensitive. "No one technique seems to have all the answers," says Weissleder. But it's too soon to tell which niche each one might fill. For now, he adds, "it's all still new."

–Robert F. Service

Additional Reading

A. Bogdanov Jr. and R. Weissleder, "The development of in vivo imaging systems to study gene expression," *Tibtech* **16**, 5 (1998).

J. Tjuvajev *et al.*, "Imaging the expression of transfected genes in vivo," *Cancer Research* **55**, 6126 (1995).

R. Moats *et al.*, "A 'smart' magnetic resonance imaging agent that reports on specific enzymatic activity," *Angew. Chem. Int. Ed. Engl.* **36**, 726 (1997).

S. Gambhir *et al.*, "Imaging of adenoviral directed herpes simplex virus type 1 thymidine kinase gene expression in mice with radiolabeled ganciclovir," *J. Nucl. Med.* (in press).