JNCI Journal of the National Cancer Institute Advance Access published January 17, 2011

Materials Science and Oncology

Nanoimaging Devices Illuminate Tumor Margins During Surgery

By Margie Patlak

In the operating room, a surgeon sees most of the tumor that needs to be removed but not the microscopic far edges, where malignant cells may be invading nearby tissue. So he or she also removes tissue surrounding the visible tumor. Later, the lab report will tell whether that surgical margin harbors tumor cells and, most important, how close the cells come to the edge of the cut. That distance can determine what happens next. If cancer cells appear all the way to the edge of the resected tissue, there is no clean margin, and the patient may have to undergo more surgery.

"The surgeon uses cutting instruments, his or her eyes and hands, intuition, and experience. No intraoperative tools or devices have successfully improved the surgeon's ability to find and remove a tumor in over half a century," noted May K. Wang, Ph.D., of the department of bioengineering at Georgia Tech and Emory University in Atlanta, and colleagues, in the 2010 issue of *Annual Review of Medicine*.

But now, researchers using nanoscale contrast agents think they can change that scenario. Devices with names like tumor paint and SpectroPen are in development, along with other advanced optical imaging systems that allow surgeons to "see" tumor cells during surgery. In preclinical studies, these agents spot fine-scale differences with a sensitivity that appears to surpass that of standard techniques, such as magnetic resonance imaging (MRI) and positron-emission tomography–computed tomography (PET-CT). None of these new imaging systems is in the clinic yet, but some have passed the proof-of-concept stage and a few are in early clinical studies.

Optical imaging systems, which typically use fluorescent dyes or proteins, have obvious advantages over standard MRI or CT scans: They are performed during surgery; they spare patients repeat surgeries; and unlike CT scans, they do not expose patients to radiation. Optical imaging systems can also scan a larger tissue area for tumor cells than pathologists can assess with the microscope. But current optical imaging systems have limitations. Subtle differences between tumor tissue and normal tissue combined with weak signaling can make it hard to see tumor margins. The hope is that the nanotechnology-based contrast agents will overcome this problem and send strong, easily detectable signals from tumor cells.

Gold Nanoparticles

Gold nanoparticles, for instance, are particularly well suited to optical imaging since they strongly absorb or scatter light. The gold metal in the particle provides a signal that is more than 100 times brighter than the fluorescent beads used in other optical imaging devices.

Wang and her colleagues have taken advantage of these properties to light up breast tumor cells, using gold nanoparticles with a Raman spectroscopy imaging system and the SpectroPen. The particles are coupled to a dye and, through an effect called surface-enhanced Raman scattering, the gold in the particle greatly amplifies the signal from the dye. The handheld SpectroPen combines a near-infrared laser and a detector for fluorescence or scattered light and is connected by a fiber-optic cable to the spectrometer that records fluorescence and Raman signals.

In a study reported in *Analytical Chemistry* in October, the researchers used the pen to detect the dye indocyanine green, infused intravenously into mice with implanted human breast cancer cells. The gold nanoparticles have a natural tendency to concentrate in tumors because they are small enough to penetrate the leaky blood vessels that feed tumors and large enough to get trapped at the edge of tumors where those blood vessels predominate.

These researchers also created another nanoparticle contrast agent, this time using the blood protein albumin, onto which they

attached a compound that fluoresces in response to near-infrared light. They tested this approach in a mouse breast cancer model, again using the SpectroPen, to precisely detect tumor margins during surgery, including those for tumors smaller than 1 mm. The SpectroPen's signal from the tumor was nearly 10 times higher than the signal from normal tissue.

According to Wang, both contrast agents may prove useful for tumor imaging during surgery, with the gold nanoparticles most effectively showing tumor cells at the edge of tumors and the fluorescent contrast agent revealing the bulk of the tumor because of its greater penetration of solid tumors. The researchers, along with colleagues at the University of Pennsylvania, are now testing the fluorescent nanoparticle in their imaging system in dogs with naturally occurring tumors, as well as in a clinical study with lung cancer patients. They also are doing basic pharmacological studies on gold nanoparticles, including biodistribution and toxicity studies, Wang said.

Other researchers have opted to home in on tumor cells with the aid of antibodies or other targeting molecules. Lissett Bickford, Ph.D., Rebekah Drezek, Ph.D., and colleagues at the Rice University department of bioengineering in Houston used gold nanoshells to which they attached a HER-2 antibody. They reported in April, in *Breast Cancer Research and Treatment*, that when they incubated their nanoshells with HER-2–positive human breast cancer tumor samples, the tumors lit up within just 5 minutes in an optical imaging system. They are currently working on creating a more portable version of the system for surgeons to use in the operating room.

"The idea with this system is that everything could be done without needing to leave the operating room," said Bickford. The hope is that "it will prevent a lot of these patients from having to have repeat procedures."

"Surgeons don't really see the difference between tumor tissue and normal tissue.. . .But these nanoparticles can paint the boundary. . ."

Combined Imaging

Other researchers are combining MRI with nanoscale contrast agents, such as fluorescent iron oxide nanoparticles. According to Lee Josephson, Ph.D., of the department of radiology at Harvard Medical School, these nanoparticles have two main advantages over standard MRI contrast agents. Their magnetic and crystalline nature heightens their ability to be detected in MRI scans. And, unlike standard MRI or fluorescent contrast agents, they are internalized by cells and are not rapidly metabolized. That means they are retained long enough so that they can be used twice: once before surgery, to reveal the approximate location of tumors with MRI, and then again during surgery, to light up the tumor boundary with an optical imaging system.

Fluorescent iron oxide nanoparticles combine the greater resolution and depth penetration of MRI with the greater sensitivity of optical imaging, said Miqin Zhang, Ph.D., of the materials science and engineering department at the University of Washington in Seattle. Without such optical imaging, "surgeons don't really see the difference between tumor tissue and normal tissue—it's like cutting in the dark. They just have an idea of where the tumor is from the MRI taken preoperatively," said Zhang. "But these nanoparticles can paint the boundary between the tumor and normal tissue during surgery, so the surgeon can precisely cut out the tumor."

Zhang and her colleagues have created the contrast agent that they call tumor paint and shown that it can cross the blood–brain barrier in transgenic mice. The nanoparticle consists of iron oxide particles labeled with fluorescence and outfitted with a peptide (chlorotoxin) that targets an enzyme overexpressed in most types of human brain tumors, as well as in prostate, skin, and colorectal cancers. As Zhang points out, accurate determination of tumor margins is especially important for brain and other tumors that are surrounded by tissue that carries out key functions. For these tumors, surgeons do not have the luxury of taking a rim of surrounding normal tissue to better ensure negative tumor margins.

The Seattle researchers reported in *ACS Nano* in July that their nanoparticle became more concentrated in tumor cells in the brain than in normal brain cells and that the difference was statistically significant. In mice, the tumors continued to light up under both optical and MRI imaging for as long as 5 days. No such contrast enhancement was seen in mice without brain tumors or in mice with brain tumors that were injected with a control nanoparticle that lacked the targeting peptide.

Encouraged by these results, Zhang's team is exploring the therapeutic use of the nanoparticles, or "theranostics," in which standard chemotherapeutic drugs and other agents are attached to nanoparticles. "These are multifunctional nanoparticles that can be used for imaging, treatment, and reporting of treatment efficacy," she said.

In Houston, the Rice researchers are also exploring theranostic strategies. In one study, they showed that they could switch their gold nanoshells from diagnostic to therapeutic use by increasing the intensity of the light the particles are showered with so that targeted tumor cells die from overheating. That study was in HER-2–positive tumors, but the ultimate goal, Bickford said, is to use the same nanoparticle to target several different proteins that tumors overexpress. The large surface-area-to-volume ratio of nanoparticles enables researchers to attach multiple targeting agents.

But the therapeutic use of nanoparticles is still primarily in preclinical studies (*see* accompanying news story). And those operating-room scenarios in which the surgeon sees accurate, lit-up tumor margins still seem futuristic at times, even to dedicated nanoparticle researchers who have yet to prove the technologies in large-scale human trials.


Miqin Zhang, Ph.D.

"It's cool having all these potential solutions under consideration," said Bickford. "I'm just curious how they will actually translate into the clinic—how realistic is it that they will actually happen."

 DOI: 10.1093/jnci/djr017