The leading edge on tumors

By Lev Osherovich, Senior Writer

Two reports by a team at the **University of California, San Diego** describe a method for visualizing tumor margins *in vivo* that could help improve the precision of surgical resection and the postoperative evaluation of resection completeness.^{1,2} To commercialize the technology, the researchers have founded **Avelas Biosciences Inc.**, which is carrying out animal studies in preparation for Phase I trials.

In the first study, the team engineered nanoparticles designed to be activated by a tumor-specific enzyme. Once activated, the nanoparticles delivered their labeling load, either a fluorescent dye or gadolinium, to the tumor cells. The label then could be detected by fluorescence or MRI, respectively.

In mouse xenograft tumors derived from human breast cancer cells, the nanoparticles preferentially accumulated in tumors instead of in nearby healthy tissue.

In the second study, the team showed that mice injected with nanoparticles carrying fluorescent dye presented well-defined fluorescent bands marking the tumor xenograft margins, which could be visualized during surgery under fluorescent illumination. Following surgery, these mice had fewer residual tumor cells near tumor resection sites and had better survival than controls that received conventional surgery.

Seeing the true edge of a tumor during resection could reduce the cost of surgical procedures by making it easy to confirm that no

tumor cells are left behind, said Quyen Nguyen, assistant professor of surgery at UCSD School of Medicine and lead author of one of the papers.

In certain solid tumors, such as prostate and pancreatic cancer, “the incomplete resection rate can be very high,” said Nguyen. “To avoid this, we currently take margins from the surgical band and send them to pathologists for analysis during the operation. This makes surgery very expensive and time consuming.”

Nguyen said that labeling tumors with fluorescent dyes could be useful in the operating theater, whereas labeling with MRI contrast-enhancing agents will help in postsurgical assessment.

Figure 1. *In vivo* tumor labeling. In two separate papers, Nguyen *et al.* and Olsen *et al.* present a platform for selective delivery of contrast agents to tumor cells to aid surgical resection and postoperative assessment.

Nanoparticles containing a payload of fluorescent dyes or MRI contrast agents are coated with polypeptides containing cationic and anionic peptide fragments separated by a protease-cleavable linker, leaving the particle with a net neutral charge [a]. Near tumors, the peptides are cleaved by locally active matrix metalloproteinases [b] to unmask a cationic peptide region [c]. The now positively charged particles bind to the tumor surface [d], delivering the payload into the cell [e].

Avelas Biosciences Inc., a new company founded by the studies' coauthors, has licensed the technology and is developing reagents for fluorescence-guided surgery.

The UCSD team was led by Roger Tsien, professor of chemistry and biochemistry. Tsien and several other authors of the studies are cofounders of Avelas.

Both studies were published in the *Proceedings of the National Academy of Sciences*.

Seeing the margin

The key challenge is selective delivery of imaging reagents to tumors rather than to healthy tissue. To achieve such specificity, the UCSD team set out to take advantage of the overproduction of matrix metalloproteinase (MMP) by tumor cells and tumor-associated stromal tissue compared with healthy tissue, which makes MMPs good markers for invasive tumor growth.³

The team engineered nanoparticles coated with a peptide consisting of a polycationic moiety, an MMP-cleavable linker and a polyanionic moiety. The immobilized peptide forms a hairpin with the two charged moieties interacting with each other to neutralize the nanoparticles. The nanoparticles can be delivered systemically because their neutral charge prevents them from being taken up by tissues before reaching the tumor target.

When the nanoparticle comes in contact with a tumor margin, the linker is cleaved by the extracellular MMPs, predominantly MMP2 and MMP9. This releases the negatively charged portion of the peptide, thus unmasking the positively charged immobilized peptide moiety, which goes on to bind negatively charged receptors on the cell surface.

The nanoparticles are subsequently internalized by the cells, where they deliver their labeling payload (see **Figure 1**, “*In vivo* tumor labeling”).

“Protease cleavage liberates the polycationic peptide at the tumor site and it then sticks to the tumor cells,” said Nguyen.

In the first study, the team showed that fluorescent dye delivered by the protease-activated nanoparticles stained tumor sites more effectively than nearby healthy tissue, and this difference in staining was greater than that seen for noncleavable peptide controls or ProSense, a tumor-specific fluorescent label marketed by **VisEn Medical Inc.** as a research reagent.

Similar results were obtained with gadolinium-carrying nanoparticles and *in vivo* MRI.

In the second study, the team showed not only that fluorescent labeling using the nanoparticles could help increase the precision of tumor resection but also that postoperative MRI of gadolinium-labeled tumors could identify tumor cells that had been missed during surgery. The latter result suggests that this labeling approach could help guide subsequent rounds of resection.

Protease power

Tito Gonzalez, VP of R&D at Avelas, said that protease activation of the nanoparticles allows selective, high-contrast labeling of tumors. According to Gonzalez, enzyme-activated labeling approaches have the advantage of signal amplification compared with approaches based purely on stoichiometric binding of peptides to receptors on

the tumor surface.

“Previous peptide-based delivery approaches had significant biodistribution and toxicity limitations,” he said. With the new nanoparticles, “the inhibitory anionic domain masks the binding properties of the delivery peptide until it’s revealed by the protease activity,” thus ensuring that labeling occurs at the tumor site.

Gonzalez said that taking advantage of the MMP activity helps zero in on the most highly aggressive part of a tumor, its leading edge, where enzyme activity is the highest and resection needs to be the most precise.

“We’re going after the cancer’s leading edge, the most active part that’s invasive,” said Gonzalez.

The UCSD team “has come up with a clever approach to differentially targeting diseased tissue,” said Kirtland Poss, president and CEO of VisEn Medical. The main novelty of the technique is the use of “compounds that have activated targeting, rather than changes in fluorescence *per se*.”

VisEn’s ProSense technology also uses tumor-associated proteases, including MMPs and cathepsin B, to convert a fluorescent dye from an inactive, quenched state to a luminous form. Activated ProSense dyes remain on the outside of the tumor, unlike the internalized labels reported by the UCSD team.

ProSense dyes are currently used as pre-clinical research reagents and are on an IND track for clinical trials in applications including cancer surgery.

According to Poss, high cathepsin B levels correlate with poor outcomes in colorectal cancer surgery, suggesting that the protease is a good marker for aggressive tumors. He acknowledged, however, that the relevant protease may vary from one tumor type to another.

“It’s hard to tell whether matrix metalloproteinases or cathepsin B will be better” as a marker of tumor growth and an imaging target, said Poss. “The answer at the end of the day will be in Phase III data.”

Poss added that the pharmacodynamics and toxicity profiles of the UCSD team’s labeling reagents will need further study.

“The key question is how these particles interact with the target of interest” in real tumors rather than in xenografts, he said.

Avelas president and CEO Paul Finnegan said the company has licensed UCSD’s patents on the technology and “must now transition from discovery to preclinical development, including formulation and manufacturing scale-up of the tumor-labeling particles toward an IND and then a Phase I trial.” The company is also running further animal studies, Finnegan added.

Finnegan noted that MMPs are also active at sites of tissue remodeling following myocardial infarction (MI) and in inflammatory bowel disease (IBD) lesions. Thus there may be other uses for Avelas’ technology beyond the company’s initial focus on cancer imaging in surgery, he said.

Meanwhile, the UCSD team is testing the labeling system in transgenic tumor models and hopes to modify the tumor-targeting nanoparticles for delivering imaging reagents and therapeutics together at the same time.

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— **Tito Gonzalez,**
Avelas Biosciences Inc.

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COMPANIES AND INSTITUTIONS MENTIONED

Avelas Biosciences Inc., San Diego, Calif.

University of California, San Diego, La Jolla, Calif.

VisEn Medical Inc., Bedford, Mass.