Nanoparticle drug delivery heats up

By Lev Osherovich, Senior Writer

Although there is broad consensus on the properties of a good therapeutic nanoparticle—the ability to concentrate a drug and release it slowly at a specific site—achieving such targeted delivery has remained elusive. Now, a pair of papers presents a blueprint for optimizing tumor targeting with nanoparticles.

One paper, published in *Cancer Cell*, describes a nanoparticle consisting of a tumor-targeting peptide coupled to a drug that is internalized by the tumor upon binding of the peptide to its molecular target on the tumor surface.[1]

The second report, in the *Proceedings of the National Academy of Sciences*, presents a two-step strategy in which tumors are first labeled with gold nanoparticles and then heated to elicit expression of a heat-related stress protein on the tumor that is subsequently targeted with a second nanoparticle carrying a therapeutic payload.[2]

Therapeutic nanoparticles encompass a broad class of carriers and drug formulations that concentrate therapeutic agents into submicroscopic aggregates and deliver them to specific tissues. In principle, nanoparticles can deliver therapeutics in a more concentrated and localized form than soluble formulations, but making a particle with the ability to concentrate, target and deliver drugs has been a challenge.

"It's advantageous to add many functions to nanoparticles, which is why they're potentially better than soluble drugs," said Erkki Ruoslahti, distinguished professor of molecular, cellular and developmental biology at the **University of California, Santa Barbara** and the **Sanford–Burnham Institute for Medical Research**. "But there's a limit to how much one can jam onto the surface of nanoparticles because one function starts competing with the others."

As described in their *PNAS* paper, Ruoslahti and collaborators at the **University of California, San Diego** and the **Massachusetts Institute of Technology** figured out that breaking up the targeting and delivery components of nanoparticles into separate, interlocking pieces actually enhances both functions.

"The key advance here is that we used two nanoparticles in concert that are more effective" than previous single-particle approaches, said Michael Sailor, professor of chemistry and biochemistry at UCSD. Sailor was the senior author on the *PNAS* paper.

Ruoslahti is corresponding author on the *Cancer Cell* paper and one of the senior authors on the *PNAS* article.

Internal affairs

Ruoslahti's team set out to find a targeting peptide to improve the delivery and tumor penetration of Abraxane nab-paclitaxel, an albumin-stabilized nanoparticle formulation of paclitaxel. Abraxane is marketed by **Abraxis BioScience Inc.** and **Otsuka Pharmaceutical Co. Ltd.** to treat breast cancer, and is in Phase III trials to treat melanoma.

In the *Cancer Cell* paper, Ruoslahti's UCSB team used phage display to identify variants of a previously characterized peptide containing an arginine-glycine–aspartic acid (RGD) motif that would increase the tumor penetration of the peptide. The motif is known to bind integrin α_V (ITGAV; CD51), which is overexpressed in tumor vasculature and therefore can be used as an anchor for delivery of a therapeutic payload to the perimeter of a tumor.

Ruoslahti's group identified RGD peptide variants that directly bound to and then were internalized by prostate tumor cells *in vitro*. In mice, the best of these RGD peptides penetrated deeper into tumors than conventional RGD peptides, which were mostly confined to the tumor vasculature.

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> ***—Erkki Ruoslahti, University of California, Santa Barbara***

The team found that internalization of the modified RGD peptide improved the delivery of Abraxane. In a mouse xenograft cancer model, Abraxane conjugated to the RGD peptide variant reached a higher concentration inside tumors and reduced tumor volume more effectively than Abraxane alone or Abraxane coupled to a conventional RGD peptide.

"The conventional RGD peptide only gets to one layer of cells around the blood vessel," said Ruoslahti. With the internalizing variant, "the payload penetrates evenly into all of the tumor."

The nanoparticle internalization described in the *Cancer Cell* paper further increases the specificity of delivery, said Chris Black, executive director of preclinical development at **Celsion Corp.**

Celsion uses local infrared heating to activate its ThermoDox liposomal doxorubicin, which is in Phase III testing for unresectable hepatocellular carcinoma and in Phase II for recurrent chest wall breast cancer.

"Internalization helps to get around the leakiness of tumor vasculature," said Black. "If you can get material into the tumor matrix and the tumor cells, you have a much more efficient targeting system" than conventional RGD peptides.

Lawrence Tamarkin, president and CEO of **CytImmune Sciences Inc.**, said the *Cancer Cell* study improves on previous efforts that relied on passive diffusion of therapeutic-bearing nanoparticles from the vasculature to tumors.

"The vascular pressure around tumors is a barrier to entry," said Tamarkin. "The challenge is how to get the therapeutic agent into a balloon." Ruoslahti's internalizable RGD variant "goes a little further than the conventional RGD" toward this goal.

CytImmune's Aurimune (CYT-6091) is a PEGylated gold nanoparticle–bound formulation of tumor necrosis factor-α (TNF-α) that has completed a Phase I trial to treat solid tumors.

Hot rod

In the *PNAS* paper, Ruoslahti joined with Sailor and used a tumor-targeting peptide called LyP1 to deliver a nanoparticle-bound therapeutic to tumors labeled with gold nanoparticles. The gold nanoparticles first were injected into tumor-bearing mice, in which they spontaneously accumulated at the tumor site by leaking through the tangled vasculature.

Next, the tumor area was bathed in infrared radiation, raising the temperature of the gold particles and the surrounding tissue.

The higher temperature induced local expression of CD8A (p32), a cell-surface protein that binds LyP1, and increased binding of fluorescently labeled LyP1 compared with that in unheated controls.

In a mouse xenograft tumor model, LyP1-bound doxorubicin reduced tumor size and increased survival compared with what was seen in unheated or LyP1-free controls.

"You've got the gold in first, identifying the tumor, cooking it a bit and making it express p32," said Sailor. "Then you bring in your LyP1-coated particle and have it stick to the tumor. This could mean that you treat patients more effectively at a lower dose" of drug.

Heating gold particles to kill tumors "has been done before, but it's the targeting that's novel," said Black.

Black noted that, unlike the denser gold particles, conventional drug-carrying nanoparticle formulations "can go anywhere in circulation, not just the tumor," eventually ending up in the liver and spleen. For this reason, previous efforts at conventional nanoparticle therapeutics have had toxicity issues, he said.

According to Black, local heating of the gold in effect turns on a cellular beacon that attracts the second, drug-carrying nanoparticle.

Digging in

Black said that, taken together, the two papers potentially solve a pair of problems in nanoparticle delivery—how to selectively target tumors with nanoparticles and how to get therapeutics bound to these particles deeper into the tumor.

However, both Black and Tamarkin noted that reproducing the results of the two-component nanoparticle system on the human scale will be a challenge.

Tamarkin said nanoparticles need to be of a uniform size and consistency to penetrate the tumor vasculature. He told *SciBX* that the Abraxane-peptide formulation used in the *PNAS* study is prone to falling apart into smaller pieces once injected and thus may be difficult to formulate correctly.

Black noted that the proteins bound by Ruoslahti's peptides are also expressed in healthy tissue, albeit at lower levels than in tumors. As a result, he said, whole-body imaging studies will be needed to confirm that the drug-carrying peptide particles go primarily into tumors.

Ruoslahti wants to confirm that the LyP1 peptide is fully internalized like the best of the RGD peptides in the *Cancer Cell* study, but he thinks the latter internalizing peptide technology is ready for clinical trials. Meanwhile, Sailor's team is developing other gold- and silica-based nanoparticles with additional functional groups that can interact more specifically with tumor proteins and drug-carrying particles.

Both researchers have filed patents on the discoveries, which are available for licensing from UCSB, UCSD and MIT.

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