Serial killing nanocarriers

By Tim Fulmer, Staff Writer

Among the challenges facing researchers in designing tumor-targeted drug delivery systems are choosing a ligand that selectively targets the tumor and consistently delivering therapeutic payload to all parts of the tumor. A group at the **University of Utah** has tackled the second task by designing a nanoscale vehicle for the delivery of chemotherapeutics that may offer improved dose regularity and tumor saturation compared with delivery systems such as polymer micelles and liposomes.

The team modified a polymeric micelle drug delivery system to create a nanogel that selectively targets tumors and can potentially distribute its therapeutic payload among multiple tumor cells. Polymeric micelles typically consist of a hydrophobic core that might serve to sequester small-molecule compounds and a hydrophilic outer shell that serves to enhance water solubility. The technique was published in *Angewandte Chemie*.[1]

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> *—Dong Shin, Emory University*

The researchers have been refining the technology over the last several years. In a previous study in xenograft mice, the group designed folate-tagged pH-sensitive polymeric micelles that improved the delivery of the topoisomerase II inhibitor doxorubicin to MCF-7 multidrug-resistant (MDR) breast cancer tumors 20-fold over free doxorubicin and 3-fold compared with polymeric micelles without folate.[2]

Tagging the micelle surface with folate helped ensure tumor selectivity, as folate receptors are overexpressed in multiple cancers. In addition, a core of pH-sensitive polyhistidine polymers allowed for cytotoxic release of doxorubicin only within acidic endosomal compartments.

The latest paper describes the next step toward designing a nanocarrier that can remain intact under pH extremes and potentially distribute its payload among multiple organelles within a cell or even between neighboring cells. To accomplish this, the polymeric micelle was modified to include an outer shell of folate-labeled bovine serum albumin (BSA), which was tethered to the pH-sensitive core via polyethylene glycol linkers.

Studies in solution showed that the tethered BSA allowed the delivery vehicle to shrink and swell in a pH-dependent manner without the disassembly that occurs with polymeric micelles under pH extremes.

Confocal microscopy of the constructs in ovarian cells showed that the reversible swelling correlated with doxorubicin release. At high pH the nanogel core was rigid. However, the core swelled when the polyhistidine residues were protonated at lower pH values, allowing the release of doxorubicin. The result was a nanoscale vehicle potentially capable of releasing chemotherapeutics in a pulsatile manner after it enters the acidic endosome of tumor cells, but not when it is in the cytoplasm and extracellular or intercellular space.

Moreover, the nanogel migrated from treated to untreated ovarian cancer cells plated on glass slides. The authors speculated that "the VM-nanogel system may be able to maximize the effect of the drug by pulsatile drug release modulated by pH and repeated entry into cancerous cells."

You Han Bae, corresponding author on the paper and professor in the Department of Pharmaceutics and Pharmacological Chemistry at the University of Utah, told *SciBX* that his lab is now investigating the nanogel's dose-dependent toxicity profile in healthy mice. The eventual goal, he said, is testing the nanogel delivery system in rodent models of MDR cancer.

Bae believes that triggering doxorubicin release when the nanogel enters the endosome and subsequent endosomal disruption could overcome drug sequestration pathways that are common in MDR cancer and could potentially provide higher concentrations of a drug to the cytoplasm and nucleus.

Although the *Angewandte Chemie* paper shows controllable drug release, **Emory University** School of Medicine researcher Dong Shin noted that "the percentage of drug released when the nanogel swells and the exact drug release mechanism and kinetics must still be determined." Shin, who is focused on tumor-targeted cancer therapies, is professor of hematology/oncology and otolaryngology.

Another unanswered question, according to Larry Tamarkin, president and CEO of **CytImmune Sciences Inc.**, is "whether the BSA outer layer, which allows the reversible swelling, is in fact invisible to the host immune system or whether it will be opsonized and cause the nanogels to be taken up by the liver and spleen before they reach the tumor."

According to Tamarkin, nanomedicines need to pass three main hurdles in animal studies, including "avoidance of the host immune system, reproducible manufacturing methodology and proof that a lower dose of chemotherapeutic delivered via nanocarrier is more efficacious than a standard dose of chemotherapeutic delivered via I.V."

CytImmune's CYT-6091, a TNF-bearing pegylated colloidal gold nanoparticle, is in a Phase I trial to treat advanced solid tumors. Following intravenous delivery, the nanoparticles preferentially accumulate in tumors as a result of leaky tumor neovasculature.

Christopher Black, executive director of preclinical development at **Celsion Corp.**, also had suggestions for additional steps, including experiments "to rule out the possibility that the so-called virus-mimetic properties are not simply the result of passive diffusion of either doxorubicin or nanogel rather than targeted migration of the vehicle."

He did say that the nanogel has potential applications beyond delivering small-molecule chemotherapeutics—for example, enzyme replacement therapy, in which the nanogel delivers enzyme directly to the

cytosol. In such cases, the bursting of the endosomal compartment by the nanogel would release a payload into the cytoplasm that might serve to improve cellular function rather than kill the cell, as happens when a chemotherapeutic is released, he said.

Celsion is enrolling patients in two Phase I trials of ThermoDox to treat nonresectable hepatocellular carcinoma and recurrent breast cancer. The compound is doxorubicin encapsulated in a heat-activated liposome. Unlike the nanogel approach, ThermoDox relies on radio frequency ablation for the tumor-specific elevation of temperature that triggers release of doxorubicin from heat-sensitive liposomes.

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