PERSPECTIVE

Shedding Light on Tumors Using Nanoparticles

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ABSTRACT The scaffold of nanoparticles (broadly defined as having a size range of 1—100 nm) presents a convenient platform to incorporate multiple functionalities into one single particle for cancer imaging and therapeutics. Whether hollow inside or not, a single nanoparticle can encapsulate a large payload of imaging probes, anticancer drug molecules, or both. On the surface, tumor-specific targeting molecules (*e.g.*, receptor-binding ligands or antibodies) may be immobilized to facilitate active tumor targeting and drug delivery. This versatile nanoplatform promises more efficient delivery of payloads to tumors for improving cancer detection and treatment.

A cohort of nanoparticles with various shapes such as quantum dots, nanotubes, nanohorns, and nanocages and made of different materials, from organic dendrimers, liposomes, gold, carbon, semiconductors, silicon to iron oxide, have already been fabricated and explored for cancer imaging and therapeutic applications (*e.g.*, see Figure 1).[1−7] However, there are various concerns associated with their use as the carrier system, including the *in vivo* safety profile, stability, drug releasing efficiency, and clearance kinetics.[1,2,8−12] Consequently, development of nontoxic biocompatible nanoparticles with favorable *in vivo* pharmacokinetics and efficient delivery to tumors is still much needed for medical applications.

In this issue of *ACS Nano*, Adair and colleagues explored calcium phosphate nanoparticles (CPNPs) as the carrier system for near-infrared fluorescence imaging of breast cancer tumors.[13] Calcium phosphate is the principle building component of hard tissues such as bone and tooth enamel in the body. Unlike most other nanoparticles, the biodegradation products of CPNPs—calcium and phosphate ions—already exist in the body at millimolar concentrations and are thus presumed to be nontoxic.

While the composition of the nanoparticles is important for cytotoxicity, other parameters like size and surface groups are mainly responsible for their *in vivo* behavior.[14] After introduction into the bloodstream *via* intravenous administration, the particles travel to the organs and peripheral tissues of the body through the blood vessels. Since the average effective pore size in normal intact endothelium is ~5 nm, nanoparticles with a hydrodynamic diameter of <5 nm rapidly extravasate out across the endothelium, resulting in a short blood circulation time.[15]

The clearance pathway is also dependent on the particle size. The glomerular filtration in kidney has a size threshold: particles with a hydrodynamic diameter (HD) of <6 nm are typically filtered and undergo renal clearance while those with a HD >8 nm do not.[12,15] Particles undergoing renal clearance will have a short blood circulation time. Large particles commonly undergo hepatic clearance in liver where Kupffer cells (macrophages located in the liver) and hepatocytes capture foreign nanoparticles for further processing: breaking down or trapping in Kupffer cells, or undergoing biliary excretion by hepatocytes.

In addition to the size, the surface groups on the nanoparticles are also critical. Depending on their surface properties such as charge and hydrophobicity, they may undergo adsorption or opsonization by serum proteins, which results in an increase in the particle size.[14] Highly charged (either positively or negatively) nanoparticles often are taken up by the macrophages in the reticuloendothelial system (RES). To minimize the nonspecific interactions and increase the nanoparticle targeting at the tumor site, a widely used method is to attach polyethylene glycol (PEG) to the sur-

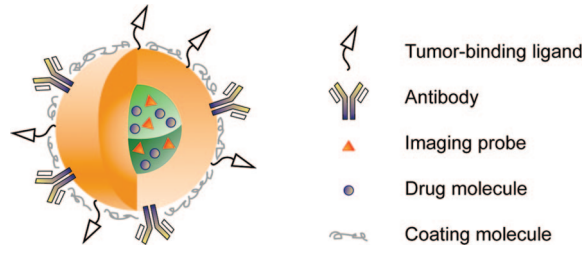

Figure 1. Schematic of a multifunctional nanoparticle with imaging probes and/or anticancer drugs encapsulated inside and tumor-specific ligands and/or antibodies presenting on the surface.

See the accompanying Article by Altinoğlu *et al.* on p 2075.

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face which can provide both steric stabilization and surface charge neutralization.

In the Adair study, the synthesized calcium phosphate nanoparticles have a spherical shape with a HD of 16 nm.[13] Carboxylate groups are present at the surface, but some (likely not all) have been conjugated with PEG to inhibit the opsonization and improve the *in vivo* pharmacokinetics of particles. Each CPNP is doped with a near-infrared dye indocyanine green (ICG), which allows real-time noninvasive imaging of the CPNP distribution and localization in living animals by *in vivo* fluorescence imaging.

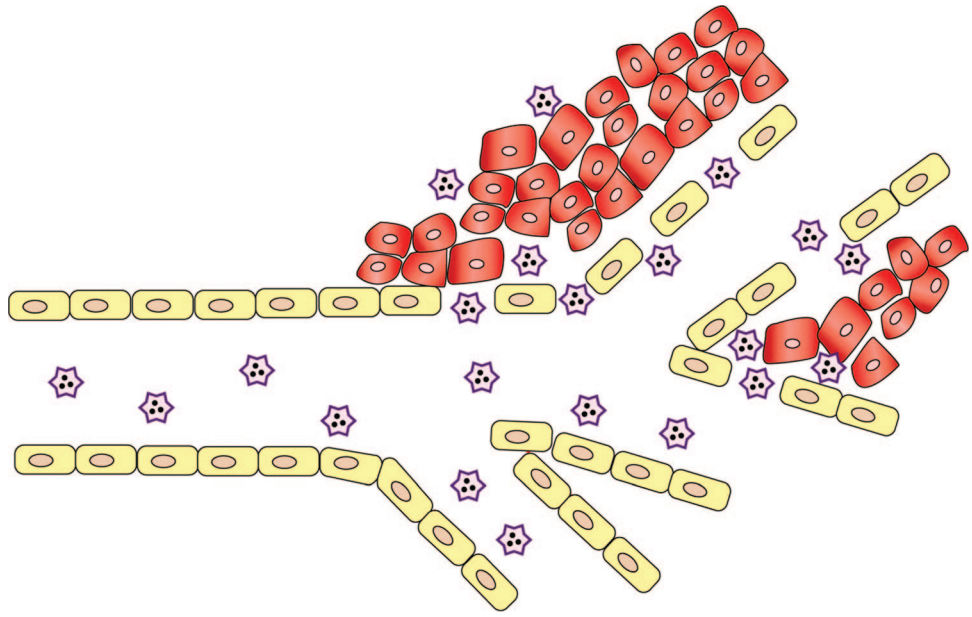

Figure 2. Nanoparticles (shown in purple) exit blood vessels (shown in yellow) in the tumor (shown in red) owing to the enhanced permeability and retention (EPR) effect. Active targeting can be further achieved with tumor-specific ligands or antibodies immobilized on the surface of the nanoparticles.

With the estimate of ~600 copies of ICG encapsulated in each CPNP, the nanoparticles have a high loading capacity. Furthermore, the authors found that each embedded ICG displayed improved chemical stability and quantum efficiency, suggesting the protective effect of the encapsulation by CPNPs. These results illustrate the advantages of using CPNPs as the carrier to deliver the imaging probes.

Adair and colleagues observed the uptake of the particles from the blood to the liver and then to the gastrointestinal tract, suggesting the mechanism of heptobiliary clearance. This clearance mechanism is different from many other nanoparticles that are taken up by Kupffer cells in RES. The heptobiliary clearance of CPNPs avoids long-term accumulation in the liver and thus limits potential hepatic toxicity.

Selective targeting of tumors with nanoparticles can be simply achieved through the well-known enhanced permeability and retention (EPR) effect (Figure 2).[16] Tumors tend to have leaky vasculature and a poorly functional lymph system. Nanoparticles with sizes of 10−100 nm may escape from the renal filtering elimination and accumulate at the tumors after prolonged circulation. The accumulation level in tumors depends on factors such as the size of nanoparticles and the leaky vascular pore, the blood circulation half-life (longer half-lives lead to higher accumulation), the degree of tumor vascularization (less accumulation in poorly vascularized tumors), and the degree of angiogenesis (poor accumulation in small preangiogenic tumors or large necrotic tumors). The last two are highly dependent on the tumor type.

Each calcium phosphate nanoparticle is doped with a near-infrared dye, which allows real-time noninvasive imaging in living animals by fluorescence imaging.

The ICG-doped CPNPs displayed much longer circulation times than free ICG, and accumulation in the xenografted breast adenocarcinoma was observed within 24 h and lasted for at least 96 h. Consequently, much brighter near-infrared fluorescence light emits from the tumors with the ICG-doped CPNPs than free ICG. This enhanced imaging signal comes from both the selective trapping of the nanoparticles (the EPR effect) and the amplification and protection effect due to the encapsulation.

It is foreseeable that the success of Adair and colleagues using CPNPs to encapsulate ICG should extend beyond cancer imaging into therapeutics, particularly for anticancer drugs that would benefit most from its protective effects, for example, small interfering RNA (siRNA) molecules with low *in vivo* stability. For therapeutics, an additional consideration is the releasing kinetics of encapsulated drugs. Relevant to this issue, a recent study on hollow calcium phosphate nanospheres may shed further insights into the capability of this system.[17] These hollow calcium phosphate nanospheres are 110−180 nm in size with shells of thickness 45 nm.

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The cavities (60 nm) are loaded with a model drug amylose. Under the irradiation of ultrasound energy, the hollow structures collapse to form pinlike nanocrystallites of calcium phosphate and the encapsulated drug molecules are released in a few minutes *in vitro*. This ultrasound-directed release of the drug payload is fast and may provide both temporal and spatial control of the releasing process.

The current set of experiments with the CPNP system relies on passive tumor accumulation based on the EPR effect. Alternatively, ligands or antibodies that bind specifically to the receptor at the tumor surface may be immobilized at the surface of the nanoparticles to realize active tumor targeting. The presence of multiple copies of tumor-specific ligands or antibodies would increase the binding avidity and enhance tumor-targeting specificity, rendering minimal drug toxicity at the normal tissues. However, the size of the nanoparticles will unavoidably increase after the ligand or antibody conjugation. Whether the resultant nanoparticles retain the same biodistribution property and clearance mechanism remains to be tested.

Similar to the CPNPs reported by Adair and co-workers, newer and more complex multifunctional nanoparticles will continue to emerge from research. These nanoparticles may contain both imaging probes and therapeutic drugs, allowing simultaneous real-time tracking of the drug location (Figure 1). A single particle may even encapsulate two or more drugs with different cancer-killing mechanisms; ligands targeting two or more different receptors on the same tumor surface may present on one single particle, which should further enhance the specificity of targeted nanoparticles. With well-designed new nanoparticles and further addition of these sophisticated multifunctions, nanoparticle-based imaging and therapeutics are beginning to have a genuine impact on cancer diagnostics and treatment in clinics.

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