Journal watch: diagnostic nanoparticles

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Magnetic nanowires for ultrasensitive detection & isolation of rare cancer cells

Evaluation of: Hong W, Lee S, Chang HJ, Lee ES, Cho Y. Multifunctional magnetic nanowires: a novel breakthrough for ultrasensitive detection and isolation of rare cancer cells from nonmetastatic early breast cancer patients using small volumes of blood. *Biomaterials* 106, 78–86 (2016).

Since metastatic disease is the most common cause of death in cancer patients [1], an early identification of circulating tumor cells (CTCs) would enable a great diagnostic and prognostic advancement. Numerous approaches have been proposed to detect CTCs, including density gradient- and size-based filtration or immuno-magnetic selection [2,3], but among their drawback are false negative or false positive results due to unspecific binding [4]. Moreover, CTCs are very rare, with roughly 1–10 CTCs being found in 1 ml of blood in a patient with metastatic cancer. New sensitive methods to detect and isolate CTCs are thus urgently needed, particularly for patients with early cancer stages.

To address this need, Hong *et al.* developed multifunctional magnetic nanowires (NW) composed of magnetic nanoparticle-doped polypyrrole, bound with a mixture of five antibodies via ss-biotin molecules (Ab-PpyNW). The authors first confirmed the efficacy of Ab-PpyNW to capture different types of cancer

cells in healthy blood samples spiked with specific numbers of tumor cell line-derived cells. After optimizing the capture conditions of Ab-PpyNW, peripheral blood of 29 nonmetastatic early-stage breast cancer patients was tested and compared with 18 healthy donors. While 5–10 ml of blood is currently necessary to identify CTCs, in this novel approach, 250 µl were sufficient to detect CTCs, whereby sensitivity increased with higher volume. A significant number of single CTCs and CTC clusters were identified in all blood samples of cancer patients analyzed, according to the stage and the received therapy, whereas in 16 of 18 healthy donors no CTCs were identified. In the remaining two healthy donors, 1–2 cells per 1 ml of blood were detected. The captured cells could easily be removed from Ab-PpyNW by glutathione-mediated release, allowing further immunohistochemical analyses. The authors have additionally developed a colorimetric technique to quickly and reliably identify the presence of CTCs in blood by adding tetramethylbenzidine to the cell suspension, which generates a color signal proportional to the number of captured cancer cells.

Multifunctional magnetic NW-based CTC isolation technique thus enables precise identification as well as ultrasensitive detection of various CTC types. By capturing CTCs from a small volume of blood, this method should advance treatment, monitoring and prognosis in numerous cancer patients.

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HDL nanoparticles for PET imaging of atherosclerosis

Pérez-Medina C, Binderup T, Lobatto ME *et al.* *In vivo* PET imaging of HDL in multiple atherosclerosis models. *JACC Cardiovasc. Imaging* 9(8), 950–961 (2016).

Atherosclerotic plaque rupture is responsible for the majority of deaths in the world. However, most of the current diagnostic modalities used for clinical atherosclerosis imaging provide limited information about plaque rupture risk or the activity of inflammatory processes. Due to its superb sensitivity, positron emission tomography (PET), an imaging technique which allows detection of nanomolar radiotracer concentrations, offers the possibility of molecular imaging of plaque progression [5].

In their paper, Pérez-Medina *et al.* utilized zirconium-89 (^{89}Zr) and the chelator deferoxamine B to label the phospholipid or apolipoprotein A-I components of the high-density lipoprotein (HDL) particles. The resulting ^{89}Zr -AI-HDL and ^{89}Zr -PL-HDL particles had size of native HDL (8.5 nm). As control, HDL particles conjugated with DiR, a near-infrared fluorescence dye were produced.

Following the detailed biodistribution studies, the authors demonstrated the accumulation of both particle types in the plaque-affected aortic regions in the *ApoE*^{−/−} mice fed high fat diet by micro-PET/CT and *ex vivo* near-infrared fluorescence, as well as in a rabbit model of balloon injury-induced aortic atherosclerosis by large animal PET/CT and PET/MRI. Further, targeting of ^{89}Zr -PL-HDL particles to the femoral plaques was confirmed by PET/CT in a porcine model of accelerated atherosclerosis induced by familial hypercholesterolemia and balloon injury. Independent of the radiolabel location on the particle, preferential uptake of the ^{89}Zr -labeled HDL particles by plaque macrophages and monocytes was observed. Importantly, the labeling with ^{89}Zr did not affect the natural behavior and distribution of HDL. In contrast, oxidized HDL particles (^{89}Zr -AI-HDL^{ox}) showed a significantly faster blood clearance in both atherosclerotic and healthy rabbits, along with increased kidney uptake. Consequently, at day five postinjection, their accumulation in atherosclerosis-affected aortas was significantly lower than that of nonoxidized ^{89}Zr -AI-HDL particles.

Although the long half-life of ^{89}Zr may prevent its use as a radiotracer in humans, the potential applicability of those particles for long-term tracking of *in vivo* HDL behavior, but also for providing insight into HDL ability to identify high-risk plaques in preclinical models is high. Additionally, the use of the HDL-like particles as a drug delivery platform, proposed by the

authors in the previous studies in mice [6] is now open to further development, as its capability of plaque targeting in models closer to human atherosclerosis has been confirmed. ^{89}Zr -HDL particles could thus constitute an attractive tool for monitoring of drug delivery to the plaque.

– Written by I Cicha

Photon roller coaster for enhancing near infrared optical imaging of tissue

Evaluation of: Levy ES, Tajon CA, Bishop TS *et al.* Energy-looping nanoparticles: harnessing excited-state absorption for deep-tissue imaging. *ACS Nano Article ASAP*, doi:10.1021/acsnano.6b03288, Web-publication: 2016–10–07.

Due to low scattering and adsorption of near infrared light (NIR) photons by tissue, for imaging purposes NIR is superior to visible light and enables imaging at a micro-scale resolution through millimeters of tissue. In their recent paper, Levy *et al.* developed a new class of materials for enhanced NIR-deep-tissue-imaging by doping β -NaYF₄-nanoparticles with lanthanides. These particles, called energy-looping nanoparticles, exploit a positive feedback loop of intermediate excited states resulting in a dramatically increased absorption of photons upon excitation at wavelengths in the NIR-II window. Additionally, this process can also lead to an upconversion of the emitted photons, which do not interfere with the dopants themselves.

To identify the lanthanide dopant compositions exhibiting the desired properties, the authors first used a high-throughput computational screening. The screening was done with materials excitable at 1064 nm, which allow the use of common lasers for the experiments. Tm³⁺ exhibited the highest rates of upconverted luminescence, with an emission at 800 nm, and could not be enhanced by co-doping with other ions. This was unexpected, since Tm³⁺ is not known to have an absorption peak at 1064 nm. The experimental tests on a library of NaYF₄-nanoparticles doped with different amounts of Tm³⁺ revealed that the energy used at 1064 nm in standard spectrophotometers is too low to produce the upconversion. In contrast, the laser of a confocal microscope was able to induce the desired effect in the range predicted by the simulations. Following a deep mechanistic study of the looping effects of, the authors performed the analyses of their imaging properties (excitation: 1064 nm; emission: 800 nm) on 1 mm phantoms and mouse brain tissue. Using 5 μm polystyrene (PS) beads loaded with the Tm³⁺-energy-looping nanoparticles, the authors showed that this new class of material is superior to the current state-of-the-art upconversion nanoparticles (excitation:

784 nm) being clearly visualized through the tissue with a resolution of approximately 2 μ m.

The authors concluded that this new class of dyes shows an improved resolution at a tissue depth of 1 mm, which is comparable to the state-of-the-art multiphoton techniques, but with significantly lower costs and infrastructure. It will be very interesting to follow, if the computational screening can discover further, perhaps even more potent dyes of this class, and how this will influence the analysis of biological mechanisms in living tissue.

– Written by S Lyer & C Alexiou

Intraoperative visualization of liver tumors using RAMAN scattering nanoparticles

Evaluation of: Andreou C, Neuschmelting V, Tschaharganeh D *et al.* Imaging of liver tumors using surface-enhanced Raman scattering nanoparticles. *ACS Nano* 10, 5015–5026 (2016).

Surgery remains the gold standard and often the only curative option in liver cancer. For complete surgical removal of a tumor and subsequent therapeutic success, a precise visualization of the tumor during surgery is crucial. Conventionally, liver tumors are detected by MRI, CT, PET or ultrasound, the techniques which have limitations in terms of intraoperative imaging and sensitivity, or are accompanied by ionizing radiation or radioactive imaging agents [7]. To address the need of a new precise intraoperative imaging technique, Andreou *et al.* explored the utility of surface-enhanced Raman scattering nanoparticles (SERS-NP) for liver imaging. To produce SERS-NPs, a reporter molecule with a specific Raman signature was adsorbed on a gold core and encapsulated in a silica shell, resulting in a final NP size of 108 nm and a zeta potential (pH 7.1) of -46.9 mV [8,9]. Based on the respective Raman reporter dye, the NP produced distinct stable spectra in serum for 24 h.

Being rich in phagocytic cells of the reticuloendothelial system, normal liver tissue rapidly takes up nanoparticles. When phagocytic cells are replaced by tumor cells, nanoparticle uptake decreases dramatically and this phenomenon can be utilized for cancer imaging. After intravenous injection in mice, SERS-NP were rapidly cleared by reticuloendothelial system. Real-time monitoring of the Raman signal showed a maximum intensity in the blood immediately after nanoparticle injection, with steady decrease due to sequestration of nanoparticles from the circulation. Accordingly, the Raman signal in the liver increased, reaching plateau within several minutes and after 12 h, tissue homogenates of livers and spleens showed a characteristic Raman signature.

To compare the localization of the nanoparticles in healthy liver and tumor tissue, analysis of the Raman

signature was supported by a direct classical least-squares model, so that normal liver tissue (Raman reference spectrum), background and cancer tissue could be clearly discriminated by use of a color scheme. Mouse models of hepatocellular carcinoma (Myc-driven) and histiocytic sarcoma (*Ink4A/Arf^{fl}*) were employed to prove the *in vivo* imaging capacity of the SERS-NP. In both models, intraoperative Raman imaging of surgically exposed livers enabled the successful detection of microscopic cancerous lesions (250 μ m), which were not detectable by MRI, visual inspection or palpation, but were confirmed by subsequent histology. Moreover, SERS-NP detected tumors with more sensitivity and specificity than indocyanine green (ICG), an established contrast agent for fluorescence imaging of liver tumors [10], due to varying tumor-to-background ratios and photobleaching of the fluorescence dye. Remarkably, SERS-NP reached a factor 40 difference in accumulation between tumor and healthy liver.

In conclusion, NP-enhanced Raman imaging can be applied for high-accuracy detection of liver tumors in mice. Although Raman imaging devices for clinical use are still under development and the regulatory approval of NP-based imaging agents might be challenging, this *in vivo* proof-of-principle study shows the clinical potential of this technique.

– Written by C Janko

Nanoparticle-based photoacoustic pH imaging & pH-responsive tumor therapy

Evaluation of: Chen Q, Liu X, Zeng J, Cheng Z, Liu Z. Albumin-NIR dye self-assembled nanoparticles for photoacoustic pH imaging and pH-responsive photothermal therapy effective for large tumors. *Biomaterials* 98, 23–30 (2016).

The microenvironment of solid tumors and healthy tissue are strongly different. In contrast to normal tissue, the hypoxia-induced neovasculature of growing tumors is often insufficient, resulting in poor perfusion, elevated interstitial fluid pressure, nutrient deficiency and severe acidity [11]. In spite of that, tumor cells can adapt to these hostile conditions, which may lead to therapeutic resistance and enhanced malignant behavior [12]. In recent years, great efforts were made to target the specific tumor environment [12,13], in particular the acidic extracellular pH, which has been found suitable for targeted therapy and imaging using pH-responsive systems [14,15].

In their recent paper, Chen *et al.* have developed pH-responsive self-assembled nanoparticles by adsorption of NIR dye croconine (Croc) onto human serum albumin (HSA) via hydrophobic interaction. The HSA-Croc nanoparticles were purified by centrifuga-

tion and ultrafiltration and characterized by transmission electron microscopy, UV-vis-NIR spectrophotometry and dynamic light scattering. The highly stable, monodispersed 100 nm HSA-Croc particles revealed low Croc release and negligible cytotoxicity even at high particle concentrations. Importantly, the absorption peaks of HSA-Croc at 680 nm and 790 nm exhibited strong and reversible pH-dependence especially in the pH-range of acidic tumor microenvironment. Thus, IR thermal images and temperature measurements of HSA-Croc solutions under the irradiation of an NIR laser showed an enhanced photothermal effect at values below pH 7.

This dual-wavelength ratiometric photoacoustic imaging and pH-responsive photothermal effect was also demonstrated in 4T1 tumor-bearing mice. An increased photothermal heating of tumor tissue and strongly reduced nonspecific photothermal heating of normal tissues were observed after intravenous administration of HSA-Croc and exposure to a NIR laser. As a result, the 4T1 tumors were completely eliminated in presence of HSA-Croc. In control mice, treated with non-pH-responsive indocyanine loaded particles (HSA-ICG), three of five tumors showed only partial reduction and continued to grow at later time points.

Due to the more acidic microenvironment of the tumor core, the resulting photoacoustic signals were much higher in HSA-Croc-injected mice than in HSA-ICG-treated controls. Consequently, the increased NIR absorbance in the tumor core led to the more effective photothermal heating of large tumors after HSA-Croc treatment.

Taken together, an intravenous injection of HSA-Croc enables the real-time visualization and diagnosis of changes in tumor pH by dual wavelength ratio-metric photoacoustic imaging. This study represents a promising novel approach to pH-responsive photothermal therapy by targeting the acidic microenvironment of the tumors.

– Written by RP Friedrich

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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