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Challenges to effective cancer nanotheranostics

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Abstract

Advances in nanotechnology for oncology will arise from an increased understanding of the interaction between nanomaterials and biological systems; refinement of multifunctional nanocomposites for applications such as simultaneous imaging and therapy (theranostics); and harnessing of the unique physicochemical properties arising from nanoscale effects which distinguish them from small-molecular-weight molecules in the detection and destruction of cancer cells with high selectivity and efficiency. The major challenges in successful clinical translation of tumor specific nanoparticle delivery include overcoming various biological barriers and demonstrating enhanced therapeutic efficacy over the current standard of care in the clinic. For many nanoparticle mediated theranostic applications, image guidance can play a crucial role not only in exploiting the cancer specific imaging capabilities of these novel particles, but in planning, targeting, monitoring and verifying treatment delivery, thus enhancing the safety and efficacy of these emerging procedures.

Keywords

nanoparticles; targeting; barriers; photothermal ablation therapy

1. Introduction

Cancer nanotheranostics is the use of nanometer-sized agents for combined diagnosis of and therapy for cancer. Integration of imaging capability into the design of nanoparticles made possible monitoring of the distribution of nanoparticles in real time. This allows tailoring of cancer treatment to individual patients by modulating the pharmacokinetics and tumor uptake of nanoparticles. Imaging also provides much needed information on the mechanism of action of nanoparticles. For example, the extent and pattern of intratumoral distribution of nanoparticles obtained in repetitive imaging sessions may be used to explain resistance or sensitivity to a particular anticancer agent delivered in nanocarriers. Some nanotheranostic agents have unique physicochemical properties that normal small-molecular-weight compounds lack. These properties can be harnessed for the use of nanotheranostics as a new method of cancer therapy.

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In spite of the promise of novel cancer nanotheranostics, significant barriers and challenges to successful clinical translation of these agents remain. 1) Efficient delivery. Without a doubt, increasing tumor uptake of nanotheranostic agents will enhance the efficiency of tumor-cell killing by those agents and reduce damage to normal tissues, thus increasing the therapeutic window. 2) Image guidance. Imaging is indispensable to cancer therapy planning as well as monitoring response to therapy. 3). Multimodal therapy. Just one treatment modality (including nanotheranostics) is unlikely to be capable of completely eradicating residual tumor cells. Therefore, the ability to integrate multiple treatment modalities in a single nanoparticle system is extremely appealing.

In this review, we discuss current challenges to efficient delivery and image guidance encountered in the use of cancer nanotheranostics. We recently reviewed topics in multimodal diagnosis of and therapy for cancer using light-activatable nanotheranostics [1].

2. Efficient delivery of cancer nanotheranostic agents

2.1. Active targeting

One of the promising features of nanomedicine is active targeting, in which homing ligands are attached to the surface of nanotheranostic agents to direct selective accumulation of them in solid tumors [2]. Over the past two decades, researchers have devoted much effort toward the development of targeted nanoparticles. However, comprehensive studies that carefully dissect various processes involved in the delivery of systemically administered nanoparticles to tumor cells, including distribution through the vascular compartment, transport across the microvascular wall, and dispersion in the tumor matrix, have been lacking. Using a simple mechanistic modeling analysis, Schmidt and Wittrup [3] hypothesized that tumor uptake of nanoparticles about 50 nm in size differed insignificantly for particles with and without targeting ligands. Also, Pirollo and Chang [4] argued that in some ligand-conjugated nanoparticle systems, tumor uptake may result from the enhanced permeability and retention effect of long-circulating nanoparticles. This raises the question of whether true targeted delivery is achieved with many of the purportedly active targeting nanoparticles. What is clear is that the tumor uptake value alone that measures the global distribution of nanoparticles in an entire tumor is insufficient to ascertain whether a particular active targeting strategy is working or not.

Targeted delivery of hollow gold nanospheres (HAuNS; ~40 nm in diameter) illustrates the importance of dissecting intratumoral distribution of nanoparticles at the cellular level. HAuNS are excellent photothermal conversion agents that are able to heat tissues in the presence of an external near-infrared (NIR) laser beam. To direct HAuNS to tumor cells overexpressing epidermal growth factor receptors (EGFRs), the anti-EGFR monoclonal antibody cetuximab (C225) was conjugated to the surface of HAuNS by our group [5]. In vitro, C225-HAuNS demonstrated selective uptake in human squamous carcinoma A431 cells that express EGFR (Fig. 1). In vivo, the distribution of C225-HAuNS in mice bearing EGFR+ A431 epidermoid carcinoma tumors was also evaluated. To quantify this distribution, HAuNS were labeled with the gamma emitter ^{111}In ($t_{1/2}=2.8$ days). Twenty-four hours after intravenous injection of C225-HAuNS into mice bearing A431 tumors, accumulation was highest in the liver due to uptake by phagocytic cells of the liver (Kupffer cells) and the fact that liver cells also express EGFRs. It also observed that the tumor uptake of the nanospheres was 48% higher than that of IgG-conjugated HAuNS, a difference that was not statistically significant (Fig. 2). However, when the data are analyzed in the context of nanoparticle distribution to the perivascular regions by counting the number of nanoparticle clusters, there was a threefold higher accumulation of HAuNS in the tumor perivascular area in mice injected with C225-HAuNS than in those injected with IgG-HAuNS (Fig. 3) [5]. Here, the number of HAuNS and their aggregates per field in the

perivascular areas in cryosectioned tissues were quantified under a dark-field microscope. These observations suggest that C225 conjugation facilitated extravasation of HAuNS into the extravascular interstitial space. However, increased extravasation of C255-HAuNS was not sufficient to lead to significantly greater tumor uptake of C255-HAuNS than of IgG-HAuNS. This is because only a small fraction of tumors had good blood perfusion. Limited diffusion of C225-HAuNS in the tumor interstitial space may restrict the dispersion of these nanoparticles away from the perivascular tumor areas. This is not surprising given that diffusion of nanoparticles is inversely related to their size and that the tumor interstitium consists of a viscous extracellular matrix and densely packed tumor and stromal cells that restrict the movement of nanoparticles [6]. Also plausible is that the binding-site barrier by which extravascular transport of nanoparticles is blocked because of strong initial binding of nanoparticles to their targets restricted the dispersion of C225-HAuNS away from the perivascular regions [7].

Given the results of the C225-HAuNS study described above, the answer to the question of whether active targeting of HAuNS nanoparticles is achieved by a receptor-mediated process depends on how the data are analyzed and interpreted. When the data are analyzed in the context of the tumor uptake value, which is normally expressed as the percentage of the injected dose of nanoparticles per gram of tissue, the introduction of homing ligands to HAuNS did not seem to make a significant difference in tumor uptake. However, when the data are analyzed in the context of nanoparticle distribution to the perivascular regions, conjugation of C255 with HAuNS resulted in active targeting of the nanoparticles to the tumors. In fact, targeted delivery of HAuNS to the perivascular regions of the tumors was sufficient to cause significant temperature elevations in the tumor tissue, modulating vascular permeability and increased delivery of macromolecular agents [8]. Similarly, in a study reported by Bartlett et al. [9], use of transferrin-targeted nanoparticles composed of cyclodextrin polycations and small interfering RNA (siRNA) may have been considered an ineffective tumor targeting strategy if the data were analyzed according to the tumor uptake value alone. In fact, although nontargeted and transferrin-targeted siRNA nanoparticles exhibited similar biodistribution and tumor localization, the targeted nanoparticles induced significantly greater RNA interference than did the nontargeted ones. This is attributed to increased internalization of the nanoparticles in tumor cells. These data underscore the importance of in-depth examination of the distribution of nanoparticles in tumors at both the tissue and cellular level.

2.2. Transcytosis as an active transport mechanism for nanoparticles

Of the many biological barriers to efficient delivery of nanoparticles to tumor cells, perhaps the least studied is the transport of nanoparticles after they evade the cells of the mononuclear phagocyte system and extravasate through tumor blood vessels. Researchers have attributed the poor tumor penetration of nanoparticles mainly to higher interstitial pressure in tumors than in the surrounding tissue (which minimizes convective transport of nanoparticles), hindered diffusion of nanoparticles owing to extracellular matrix components and tightly packed stromal and tumor cells, and binding-site barriers [10-13]. Two approaches overcoming these difficulties have been investigated. The first approach, which is discussed in below, is to use an active transport mechanism, whereas the second approach is to reduce the barrier to nanoparticle delivery imposed by the extracellular matrix.

Using melanocortin type-1 receptor overexpressed in melanoma cells as a model target system, we have shown that uptake of HAuNS conjugated with the melanocortin type-1 receptor agonist peptide NDP-MSH was almost three fold higher than that of PEGylated HAuNS (PEG-HAuNS) in B16/F10 melanoma cells. Surprisingly, we observed markedly greater dispersion of NDP-MSH-conjugated PEG-HAuNS than of PEG-HAuNS in the tumor matrix [14]. To confirm our findings, we investigated the nature of targeting ligands

(i.e., agonists versus antagonists) to determine whether it affects extravascular transport of HAU_{NS}. We found that agonist peptide-conjugated HAU_{NS} but not antagonist peptide-conjugated HAU_{NS} were internalized by B16/F10 cells [15]. Moreover, using a multilayer tumor-cell model and transmission electron microscopy, we concluded that agonist-conjugated HAU_{NS} but not antagonist-conjugated HAU_{NS} underwent transcytosis, resulting in efficient transcellular transport. We confirmed this finding in vivo, observing that whereas only small amounts of PEG-HAU_{NS} and antagonist-conjugated PEG-HAU_{NS} were localized primarily in the perivascular tumor area, agonist-conjugated PEG-HAU_{NS} were distributed throughout the tumor volume at significantly increased quantities [15].

These results showed that use of the active transport machinery (i.e., receptor-mediated transcytosis) to move nanoparticles through densely packed tumor cells to achieve improved tumor-targeting efficiency is feasible. Clearly, a more detailed mechanistic understanding of particle transport through the tumor matrix is needed to facilitate design of efficient delivery systems for anticancer nanoparticles.

2.3. Pharmacological and physical approaches to intratumoral dispersion of nanoparticles

In addition to the use of biological processes to facilitate intratumoral dispersion of nanoparticles, investigators have used pharmacological and physical methods to facilitate extravasation and interstitial dispersion of nanoparticles. For example, Lu et al. [16] showed that tumor priming by intravenous injection of paclitaxel induced apoptosis in subcutaneously inoculated human FaDu pharyngeal tumors, expanded the tumor interstitial space, and promoted delivery and interstitial dispersion of nanoparticles. Also, degradation of the extracellular matrix by collagenase or hyaluronidase increased the distribution of a monoclonal antibody and liposomal doxorubicin in tumor twofold to fourfold [17, 18]. Furthermore, tumor penetration of nanoparticles less than 100 nm in diameter with collagenase increased in a multicellular spheroid model [19]. The use of collagenase or hyaluronidase is not practical, however, because these enzymes would also damage matrix in normal tissues. Research directed at overcoming the barriers to drug delivery has led to testing of losartan, a clinically approved angiotensin II receptor antagonist. Recent studies have shown that losartan is an effective antifibrotic agent. Diop-Frimpong et al. [20] found that losartan inhibited collagen I production by tumor-associated fibroblasts, which led to a dose-dependent reduction in stromal collagen in desmoplastic models of human breast, pancreatic, and skin tumors in mice. As a result, the drug enhanced the efficacy of intravenously injected pegylated liposomal doxorubicin (Doxil). Studies of preclinical murine pancreatic tumors have demonstrated a synergistic inhibitory effect of gemcitabine and an angiotensin type-1 receptor blocker [21]. A retrospective study of patients with pancreatic cancer receiving gemcitabine and angiotensin I-converting enzyme inhibitors or angiotensin II type-1 receptor blockers showed improved clinical outcomes in these patients [22]. These studies indicated that pharmacological modulation of the tumor extracellular matrix to increase the delivery of nanoparticles is a viable approach to treatment of cancer with nanocarriers, and further clinical studies combining approved nanomedicine and clinically-used angiotensin inhibitors are warranted.

Regarding physical approaches, various external energy sources are used to modulate tumor vascular permeability and increase interstitial dispersion of nanoparticles. For example, using core-shell nanoparticles composed of superparamagnetic iron oxide (SPIO) cores and gold nanoshells (SPIO@AuNS), Melancon et al. [23] demonstrated an approximately twofold increase in the number of SPIO@AuNS delivered to tumors in the presence of an external magnet over that delivered to tumors without the magnet after intravenous injection of the nanoparticles. In addition, Kong et al. [24] showed that tumor hyperthermia (<42°C) enhanced the delivery of nanoparticles to tumors. Similarly, a photothermal effect mediated by C225-HAU_{NS} and NIR light increased vascular perfusion in tumors and enhanced the

delivery of a polymeric drug injected at the time of initiation of laser treatment [8]. How these various techniques will be implemented in future clinical applications of anticancer nanomedicine remains to be determined.

3. Image guidance for cancer nanotheranostics

3.1. Pharmacokinetics and biodistribution

Imaging permits a non-invasive means of visualizing nanoparticles in intact *in vivo* systems in order to elucidate mechanisms of action of drug-loaded nanoparticles, which may be different from those of free drugs. Noninvasive imaging also enables longitudinal assessment of the biodistribution as well as mechanisms of clearance of nanoparticles from the body. This can aid in addressing critical questions, such as specificity of nanoparticle uptake, the rate of uptake, the distribution of material within the heterogeneous tumor environment, and the rate and routes of clearance from the target site. Such information is invaluable in patient selection and stratification for nanoparticles-based anticancer therapies.

In recent years, multimodal imaging has emerged as an indispensable noninvasive tool for determining the efficiency of nanoparticle delivery. For example, Medarova et al. [25] described the use of magnetic resonance (MR) imaging (MRI) and fluorescence imaging to monitor the local tumor accumulation and functional activity of magnetic nanoparticles covalently linked with siRNAs. That study illustrated the power of using multimodality imaging approaches to correlate localization of therapeutic agents with their biological activity. Also, Bartlett et al. [9] examined active targeting of nanoparticles formed with cyclodextrin-containing polycations and siRNA by transferrin with positron emission tomography and bioluminescent imaging using ^{64}Cu -labeled siRNA nanoparticles. They observed an increase in cellular uptake of the nanoparticles in tumor cells rather than overall tumor localization of the nanoparticles.

One of the benefits of nanotechnology is that it has multiple capabilities that small molecular compounds do not, in particular, integration of diagnostic and therapeutic capabilities in a single nanodevice. This allows for tailoring of nanomedicine to individual patients to ensure successful delivery and detection of nanoparticles at disease sites. Combining diagnosis and therapy into one process, referred to as cancer nanotheranostics, makes the see-and-treat strategy possible for cancer patients. Various imaging reporters (e.g., radiotracers, fluorophores/absorbers, T1/T2 magnetic resonance contrast agents etc.) have been incorporated into nanoparticles designed to facilitate *in vivo* visualization of nanoparticles [26-32]. For example, gold nanoshells were encapsulated with SPIO and used for MRI-guided photothermal ablation therapy (PTA) [31], CuS nanoparticles were labeled with ^{64}Cu for positron emission tomography-guided PTA therapy [32], and HAuNS were used for photoacoustic tomography-guided PTA therapy taking advantage of their intrinsic high absorption in NIR regions [26].

For cancer therapies mediated by external energy sources (such as ionizing or near-infrared radiation), nonspecific accumulation of nanoparticles may result in damage to normal tissue. This risk can be minimized by incorporation of image-based treatment planning based on *in vivo* distribution of nanoparticles and patient-specific anatomy. For energy based therapies, the decision to proceed with an intervention critically relies on knowledge of the selectivity of nanoparticle delivery. While nanoparticles can be made visible by standard imaging techniques, such as MRI or PET, for tumor localization, researchers have yet to exploit imaging techniques that allow simultaneous interrogation of the distribution of both nanoparticles and drugs delivered by these nanoparticles.

3.2. Pharmacodynamics and treatment response

A case of study where imaging plays a critical role in cancer treatment is nanoparticle-mediated PTA therapy. The use of thermal ablation in the management of cancer has grown tremendously over the past decade. Thermal ablation uses heat to directly destroy tissue. Many different energy sources are used in this procedure, including radiofrequency ablation, high-intensity focused ultrasound, microwaves, and lasers [33-37]. Energy can be provided by external and internal means via interstitial, intraluminal, and intracavitary approaches [38]. Thermal ablative therapy, in particular radiofrequency ablation, has been successful in regions where real-time monitoring of heating isn't critical. This has been primarily liver and bone, with applications in kidney, lung, prostate, breast, etc. not enjoying the same degree of success or acceptance. In several of these sites, cryotherapy is becoming attractive. One of the reasons for this is likely that cryotherapy delivery can be monitored to assess the extent of treatment. Primary barriers to PTA in the clinic have been that laser heating is relatively fast and the extent of heating is dependent on local tissue properties, such as perfusion of nearby vessels. Therefore, PTA safety and efficacy is more dependent on a monitoring modality. To this end, laser ablation systems which specifically incorporate MR temperature imaging guidance are appearing on the market to complement the existing equipment cleared for laser ablation.

Image-guided thermal ablation of tumors is a minimally invasive technique that can be performed using repeatable low-impact procedures that reduce the severity of adverse complications and postoperative tissue morbidity without adversely impacting outcome. These procedures are often associated with less soft-tissue trauma and blood loss than conventional surgery and can often be performed in outpatient settings with minimal anesthesia and numbers of support personnel. Because of these benefits, image-guided thermal ablation of tumors is extremely attractive to both patients and health care providers. In PTA therapy, high-power photo-energy is delivered via laser fibers placed interstitially in the tumor. Multiple laser fibers can be placed in the treatment volume, and unlike with other interstitial heating techniques, they can be fired simultaneously to rapidly treat large volumes of tissue. Modern PTA systems use compact high-power laser diode systems with actively cooled applicators to help prevent tissue charring during treatment.

Whether thermal therapy causes cell death and tissue damage depends on the magnitude and duration of temperature elevation. Such thermal damage can be predicted using Arrhenius analysis [39, 40] or the Sapareto-Dewey isoeffect thermal dose relationship [41]. Using the latter, the thermal dose is calculated in equivalent time of exposure at 43°C (t_{43}) using the following formula:

$$(t_{43})_N = \sum_{i=1}^N R^{(43^\circ\text{C} - T_i)} \cdot \Delta t, \text{ with } R = \begin{cases} 0.25, T_i < 43^\circ\text{C} \\ 0.50, T_i \geq 43^\circ\text{C} \end{cases}, \quad [\text{eq.1}]$$

in which Δt is the time in minutes between temperature estimates (i.e., the time elapsed between measurements of subsequent temperature), T_i is the tissue temperature in degrees Celsius for the i th measurement, and R is an empirically derived constant taken from previous high-temperature hyperthermia experiments with living tissue and extrapolated to thermal ablation experiments. This model has been extensively validated in in vivo systems and used to demonstrate that within defined temperature ranges, thermal damage is approximately linearly dependent on exposure time and exponentially dependent on temperature elevation [42].

Temperature measurements in thermal ablation therapy are typically performed with invasive fluoroptic probes, but this method is highly variable. In comparison, MR

temperature imaging (MRTI) is a noninvasive method that qualitatively and quantitatively characterizing tissue temperature changes [43, 44]. Progress in MRTI has facilitated noninvasive assessment of thermal damage to target volumes [45]. Several MR parameters are temperature-sensitive, including relaxation time (T1 and T2), bulk magnetization, and proton resonance frequency. The proton resonance frequency in particular is the most sensitive of these three parameters to temperature and is most commonly used to monitor temperature changes during thermal ablation, with temperature errors limited to 0.5-1.0°C [46-48]. Tissue temperature is directly related to proton (spin) mobility in tissues [49]. Both the T1 and apparent diffusion coefficient techniques have tissue-dependent temperature-sensitivity coefficients, requiring users to calibrate the target tissue to perform useful thermometry. Also, as irreversible damage to the target tissue occurs during thermal ablation therapy, both T1 and apparent diffusion coefficient measurements deviate from a linear response to temperature [50-52], making them better suited for qualitative monitoring at high temperatures. For qualitative imaging, T1-weighted MRI has been quite effective in guiding thermal ablation procedures for tumors in moving organs such as the liver [53] and as an aid in monitoring treatment in the clinic [54, 55]. Additionally, T1-weighted MRTI may be the best option for monitoring temperature changes in adipose tissue [56].

Despite its benefits, MRI-guided PTA is in its infancy as a cancer treatment modality. Equipment and techniques as well as preclinical validation and commercial availability of integrated PTA systems should be improved to hasten the use of this technique in humans [57]. For example, MR-compatible bone and soft tissue coaxial needles and MR-oriented stereotactic guidance equipment should be made available and implemented in the clinic. Furthermore, the availability of highly parallel multicentral processing units and multigraphics processing units as well as unprecedented exascale computing systems that facilitate real-time modeling and simulation of MR-guided PTA using finite element modeling will be possible in the future. Fusing patient-specific modeling of laser heating over a complex heterogeneous volume of tissue with MR-based temperature monitoring has great potential to be clinically useful. Use of computational models and simulation for prediction of laser-induced bioheat transfer may be exploited for prospective treatment planning for, real-time model-assisted monitoring of, and even real-time control of MR-guided PTA procedures [58, 59].

While image-guidance techniques such as MR-based temperature imaging aid in quantitatively assessing the delivery of some nanoparticle mediated therapies in vivo, significant challenges in other areas remain. Direct or indirect imaging techniques for monitoring activation of nanoparticles need to be explored. For example, noninvasive imaging methods to assess specificity of surface receptor binding, endocytosis of nanoparticles, modulated drug released (distinguishing between free versus nanoparticle-bound drugs) are needed.

4. Conclusions

Oncology is one of the disciplines that have benefited the most from nanotechnology. A wide acceptance of cancer nanotechnology will come from a better understanding of how nanoparticles interact with biological systems; how multiple functions, including imaging and therapy, can be incorporated in a single nanopatform; and how to harness the unique physicochemical properties of nanoparticles that do not otherwise exist in small-molecular-weight molecules for the detection and destruction of cancer cells with high selectivity and efficiency. Whereas researchers have successfully introduced a few nanotechnology-based anticancer therapeutic agents, such as liposomal doxorubicin (Doxil) and human serum albumin-bound paclitaxel (Abraxane), into the clinic, targeted delivery of nanoparticles for treatment of cancer has yet to live up to its promise. The major challenges in successful

clinical translation of targeted delivery of nanoparticles include overcoming various biological barriers and demonstrating better therapeutic efficacy than that of the current standard of care in the clinic. Understanding these challenges is imperative for effectively moving the field of cancer nanotheranostics forward.

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Fig. 1.

Selective binding of anti-EGFR-conjugated HAuNS to A431 cells. A431 cells were seeded onto a 96-well plate and incubated with C225-HAuNS (7.3×10^{10} particles/mL), IgG-HAuNS (7.3×10^{10} particles/mL), or C225 (500 μ g/mL) plus C225-HAuNS for 30 min at 37°C. Only cells incubated with C225-HAuNS had a strong light-scattering signal. Cells were stained with DAPI for visualization of cell nuclei (*blue*). Light-scattering images of nanoshells were pseudocolored green. Original magnification, $\times 630$. Reproduced with permission [5].

Fig. 2.

Biodistribution of ^{111}In -labeled DTPA-C225-HAuNS and DTPA-IgG-HAuNS. The mean uptake of ^{111}In -DTPA-C225-HAuNS in the liver ($\pm$ standard deviation [SD]) was significantly higher than that of ^{111}In -DTPA-IgG-HAuNS ($p=0.001$). Also, the mean tumor uptake of ^{111}In -DTPA-C225-HAuNS ($\pm$ SD) was higher than that of ^{111}In -DTPA-IgG-HAuNS, but the difference was not statistically significant ($p=0.08$; $n=4$ /group of mice). Reproduced with permission [5].

Fig. 3.

Intratumoral distribution of hollow gold nanospheres (HAuNS). (A) Dark-field microscopic images of the perivascular areas in slices of A431 tumors in mice injected with C225-HAuNS and IgG-HAuNS. HAuNS were pseudocolored green. Cell nuclei were stained with DAPI (blue). (B) Graph of the mean particle counts per viewing field ($\pm$ SD) under the dark-field microscope (magnification 200 $\times$; $n=5$ /group of mice; $*p<0.01$) compared with C225-HAuNS. Reproduced with permission [5].