Nanotechnologies in Cancer Treatment and Diagnosis

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Abstract

Despite significant efforts toward research and treatment development, cancer continues to be a major health problem in the United States that is only further enhanced by the heterogeneous nature of the disease. Nanotechnology has evolved as a technology with applications to medicine and the potential to improve clinical outcomes, with its application to cancer garnering much attention recently. In particular, through the generation of novel nanoscale devices and therapeutic platforms, nanotechnologies have emerged as innovative approaches that enable the detection and diagnosis of cancer at its earliest stages, and the delivery of anticancer drugs directly to tumors. This article highlights recent advances in the development of nanotechnologies for cancer therapeutics and diagnostics, and focuses on the potential future of cancer nanotechnology and the challenges this young field faces as it continues to move toward clinical translation. (*J Natl Compr Canc Netw* 2014;12:1727–1733)

Combined with surgical resection, chemotherapy and radiation remain the first line of treatment for patients with cancer. Improvements have been made to chemotherapies, but many drugs are still not reaching the tumor site at effective doses, and are often associated with high systemic toxicities and poor pharmacokinetics. Moreover, for many malignancies, diagnosis is achievable only at late, metastatic stages of development, reducing the

overall effectiveness of treatment. In recent years, the field of nanotechnology has emerged as an approach with the potential to produce novel diagnostics and therapeutics. At the nanoscale, materials are comparable in size to biological entities. They exhibit the ability to traverse the cellular environment in a size-dependent fashion, and can overcome a variety of biological obstacles, such as the blood-brain barrier and skin.^{1–4}

In its application to cancer, the advantages of nanotechnology are numerous and include the selective targeting and delivery of anticancer agents to tumor tissues, and devices for early cancer detection and imaging systems. Significantly, these approaches can be used to enhance tumor regression by delivering multiple types of therapeutics, or can be used to monitor therapeutic efficacy by combining therapeutic and imaging agents in a single multifunctional platform (Figure 1).^{5,6} As part of the National Nanotechnology Initiative, NIH has invested more than \$400 million dollars in 2012, with budget proposals comparable for 2013 and 2014 to continue nanotechnology-based biomedical research.⁷ Additionally, several phase I and II clinical trials of anticancer nanotherapeutics are currently underway, suggesting that the field of cancer nanotechnology and its translation are moving forward.^{8–11} However, nanotechnologies in cancer have yet to obtain mainstream use in clinical care, indicating that much progress remains to be made in the area of translation. This article briefly reviews recent advances in the development of cancer nanotechnologies, and discusses the potential future directions and challenges of this new field.

Nanotechnology-Based Platforms for Cancer Therapy and Diagnosis

Through either encapsulating or conjugating existing chemotherapeutics to their surfaces, the traditional use

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Submitted July 12, 2013; accepted for publication August 1, 2014.

The authors have disclosed that they have no financial interests, arrangements, affiliations, or commercial interests with the manufacturers of any products discussed in this article or their competitors. The content of this publication neither reflects the views or policies of the Department of Health and Human Services, nor does its mention of trade names, commercial products, or organizations imply endorsement by the US government.

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Figure 1 Schematic of multifunctional nanoparticle platform. The surface (corona) of the nanoparticle can be functionalized with targeting molecules, imaging agents, therapeutic drugs, and hydrophilic polymers, such as polyethylene glycol (used here to shield the nanoparticle from immune clearance). The core interior can be solid or hollow, depending on the material. Hollow cores can be used for the encapsulation of imaging and therapeutic agents.

From McNeil SE. Nanotechnology for the biologist. *J Leukoc Biol* 2005;78:586, published with permission of the Federation of American Societies for Experimental Biology.

for nanotechnology in cancer therapy has been to make established drugs new again through nanoformulations. The primary appeal is that nanosized carriers can increase the delivered agent's overall therapeutic index. This capability is largely due to their tunable size and surface properties; for example, the addition of polyethylene glycol to carrier surfaces can increase circulation time through reducing renal and immunologic clearance, and, in turn, increases their accumulation at the site of action (Figure 2).¹² Furthermore, with optimized design, the timing or site of drug release can be controlled by material composition or via a triggered event, such as light exposure, ultrasound, or change in pH.^{13–18} Nanoparticles that expand and slowly release their chemotherapeutic cargo when exposed to acidic pH have been designed for release in the endosome after cellular uptake.¹⁷ However, the value of nanomaterial-based delivery has become more apparent for new types of therapeutics, such as those using nucleic acids (ie, DNA, RNA), which are highly unstable in systemic circulation and sensitive to degradation in their free form. Nanoparticle-conjugated, gene silencing, short interfering RNAs (siRNAs) have been reported to

have an extended half-life 6 times longer than free RNA.¹⁹ Further, the increased stability of nanocarrier-delivered siRNA often combined with controlled, continued release has been shown to prolong gene silencing effects.^{20–22}

Size is a major factor in the targeted delivery of nanotherapeutics to cancerous tissues, and hence their ability to enhance therapeutic efficacy. Selective delivery of nanotherapeutic platforms depends primarily on the passive targeting of tumors through the enhanced permeability and retention (EPR) effect. This phenomenon relies on defects in lymphatic drainage, along with increased tumor vasculature permeability, to allow nanoparticles (optimal range, 70–200 nm) to leak into and accumulate in the tumor microenvironment.^{6,23,24} Unfortunately, the drawback to reliance on this mechanism of targeting is that it is primarily limited to solid tumors, and is less ideal for targeting small, poorly vascularized metastases, making it highly dependent on the size and shape of the nanocarrier and tumor type.²⁵ Moreover, limited data exist on the effectiveness of enhanced drug accumulation via EPR in humans. With this in mind, researchers are moving toward engineering nanoparticles that actively target tumor cell surface markers. A variety of ligands can be used to target tumor cells or the tumor microenvironment, such as aptamers, antibodies, growth factors, and small molecules (Figure 2). Several studies have shown that active targeting and receptor-mediated internalization are necessary for genetic therapies, and can increase the anticancer activity of the associated drug, with significant potential for translation to the clinic.^{6,10,12}

However, targeted nanotechnology-based platforms have yet to fully make the transition from concept to clinical translation. The reasons for this are multifold, but primarily stem from concerns regarding consistency in reproducing targeted nanoparticles with the same ligand density and activity, reducing nonspecific biomolecule interactions, and optimizing the ligand density with surface marker expression on tumor cells, which may be highly variable.^{26–28} Several ligand screening and selection strategies are being designed to overcome these challenges.^{29–31} Nonetheless, the combination of passive and active targeting is what may enhance the delivery of nanotherapeutics. Previous work suggests that the function of EPR in particle localization is in its

Figure 2 Chemical and physical properties of nanomaterials. Displayed are characteristics that affect nanomaterial performance in vivo and in vitro. Abbreviations: +, proton; -, electron; NH₂, amino group; NPs, nanoparticles; R, carbon-containing group; COOH, carboxyl group; SH, sulfhydryl group; OCH₃, methoxy group.

Adapted from Kamaly N, Xiao Z, Valencia PM, et al. Targeted polymeric therapeutic nanoparticles: design, development and clinical translation. *Chem Soc Rev* 2012;41:2993, published with permission of The Royal Society of Chemistry.

capacity to facilitate the accumulation of nanocarriers at the tumor site, whereas targeting ligands play an important role in retention and cell uptake.^{32–34} Accordingly, it seems that shape, size, and targeting are all significant factors in the effectiveness of a therapeutic and should be considered in the design of optimal nanosized delivery vehicles.

In most nanoformulations, lipid- and polymeric-based nanomaterials are used because of their biological compatibility. The range of nanomaterials in development for use in nanotechnology-based therapeutics is expanding and includes metal iron oxides (superparamagnetic particles), gold (nanoshells, spheres, rods), silica (mesoporous particles), DNA/RNA (DNA origami, RNA nanoparticles), and carbon (nanotubes, fullerenes).^{20,35–39} In some cases, these nanomaterials are creating new cancer treatments, such as hyperthermia and photothermal therapies. Iron oxide nanoparticles can be heated in an applied alternating magnetic field, precisely damaging and killing surrounding tumor cells, whereas gold nanoshells (and gold rods/spheres) respond to near-infrared light exposure by releasing energy in the form of heat.^{37,40} Importantly, thermal ablation can be combined with therapies such as radiation and chemotherapeutic drug release from nanoparticles. Both combinations have been

shown to sensitize and enhance the overall therapeutic effect on tumors, supporting the expansion of nanotechnology-based therapeutics as a platform for combined therapies.^{15,41,42}

In addition to their role in drug delivery, nanomaterials have been used to detect and diagnose early-stage cancers, identify metastases noninvasively, and guide tumor removal during surgery. For image-guided surgery, the use of targeted nanoparticles to identify lesions has been an active area of investigation. In general, targeted nanoparticles can provide molecular imaging functionality through site- or activity-specific localization. Iron oxide nanoparticles provide a sensitive, low-toxicity alternative to standard MRI contrast agents, such as injected gadolinium.³⁹ Quantum dots—nanoparticles with size-tunable light emission—can also be used to image and guide accurate tumor removal, in addition to other strategies shown to be viable options in vivo.^{43–45} Further, using nanotechnology, the potential exists to develop multimodal systems that join several imaging techniques into a single platform, allowing for varied analyses of tumor anatomy. This is clearly demonstrated by the Gambhir laboratory, which has developed a triple-modal nanoparticle consisting of a gold core coated with a Raman molecular tag and gadolinium (Figure 3).⁴⁶ Using this platform, the in-

investigators were able to precisely visualize tumors and extending metastases in the brain using photoacoustic, Raman, and magnetic resonance imaging. New strategies are also underway to extend multimodal imaging at the individual-cell level for nanoparticle-mediated cell labeling and tracking.⁴⁷

Devices using nanotechnology for noninvasive early detection or treatment monitoring are also highly sought after. Highly specific and sensitive in

vitro diagnostics, such as multiplexed protein and nucleic acid nanosensors, detect multiple biomarkers at low concentrations, improving the detection of stage-specific tumor signatures. Gold or magnetic particles can easily be conjugated to nucleic acids or proteins for use as biomarker probes or labels, and can measure biomarkers directly from bodily fluids.^{48–50} Moreover, many of these devices have the potential for use as point-of-care diagnostics at low cost without losing sensitivity (clinical sensitivity, 84%–89%).^{51–53} They can further be used to capture circulating tumor cells and monitor biomarker expression in response to therapy.^{50,54,55} It should be noted that the worth of these devices is highly dependent on the measured markers, highlighting the importance of biomarker discovery and validation by cancer biologists in partnership with nanotechnology development.

Figure 3 Detection of brain tumors in living mice by triple-modality MRI-photoacoustic-Raman nanoparticle. (A) Top panel shows a schematic of triple-modality MRI-photoacoustic-Raman nanoparticle (MPR) and the intravenous injection into mouse with an orthotopic brain tumor. Bottom panel shows a concept of clinical use in which a single injection will allow the detection of tumor presurgery, during surgery, and postsurgery using each of the 3 modalities. (B) Two-dimensional axial MRI, photoacoustic, and Raman images of mouse brain tumors before and after MPR injection. (C) Three-dimensional rendering of MRI and photoacoustic (PA) images of tumor with an overlay of both image types in the bottom panel. (D) Quantification of imaging signals. N=4 mice. Data represent mean $\pm$ SEM.

*** $P < .001$.

** $P < .01$ (1-tailed t test).

Abbreviation: AU, arbitrary units.

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Future of Nanotechnology in Cancer

Multifunctional and Smarter Nanotechnology-Based Platforms

As more basic capabilities are mastered in nanoparticle design, combined functionalities will likely become more prevalent. Theranostics, the joining of therapeutics, diagnostics, and often posttherapy monitoring, is a new area of interest in nanotechnology. By design, metallic and magnetic nanomaterials, such as gold and iron oxide, respectively, have proven to be ideal for these types of applications; both display imaging properties (ie, luminescence and magnetic resonance) and can be designed to serve as nanocarriers.⁵⁶ In particular, Lee et al⁵⁷ recently described the functionalization of iron oxide nanoparticles with a targeting moiety and chemotherapeutic drug specific for pancreatic cancer. The iron oxide nanoparticles not only were able to be detected by MRI on initial accumulation at cancer sites but were also detectable several days later, serving as a monitoring tool for drug-resistant tumors. Similarly, another study showed that targeted nanoemulsions containing iron oxide crystals, a fluorescent dye, and therapeutic could be used to image nanoparticle accumulation in a colon cancer mouse model and deliver drugs that impeded tumor growth.⁵⁸ Irrespective of their composition, nanocarriers in general can be designed to contain multiple agents and materials to expand their functionality.^{3,59} For example, a plat-

form with mesoporous silica and liposome characteristics was recently developed and shown to enable targeted delivery and the controlled cytosolic release of various cargoes encapsulated within its core, including imaging and therapeutic agents.⁵⁹

The means through which nanotechnology can be applied to cancer research and treatment are proving to be extensive. This is noticeably demonstrated in the use of nanodevices for single-cell proteomic studies of therapeutic response and in nanopore-mediated genomic sequencing, which has the potential to dramatically increase throughput and lower the cost of sequencing.^{55,60,61} Most recently, an increase has occurred in the integration of nanotechnology and cancer biology discovery, creating a path to novel cancer target validation. Cancer biologists are identifying hundreds of potential drug targets that could play a vital functional role in cancer progression, while many nanotechnologists have developed readily modifiable platforms for anticancer agent delivery. Working together, nanotechnologists and cancer biologists are taking the extensive data produced through genomics and, after *in vitro* analyses, evaluating potential targets and treatments *in vivo* through mouse models using nanotechnology-based delivery systems.^{62,63} Thus, apparent points exist at which nanotechnologists, cancer biologists, and clinicians can and are collaborating to propel cancer target discovery.

With the development of more complex nanosystems, opportunities seem to exist to further evolve cancer nanotherapeutics away from reformulations and to use their properties in the development of smarter, biologically responsive nanosystems. For example, von Maltzahn et al⁶⁴ recently developed nanotherapeutics able to communicate with one other in response to the activation of a biological cascade to signal for increased accumulation at tumor and metastatic sites, whereas another nanosystem takes advantage of multiple marker expression on cancer cells to regulate payload release at the appropriate location.³⁵ This type of work is further extending to proof-of-principle studies of remote-activated protein-producing nanoparticles that could potentially be used to produce drugs at the site of action.⁶⁵ However, not all nanosystem designs are so far-reaching, and can be as simple as mimicking the naturally occurring cells and molecules in the body (eg, coating of nanoparticles with cell membranes

and cell-specific markers that prevent phagocytosis) as a way to outwit the immune system, providing another avenue for smarter nanopatform design with increased utility in the body.^{66–69}

Optimization of Particle Design: Importance of *In Vivo* and *In Vitro* Characterization

The ability to control the chemical and physical properties of nanomaterials is essential in the regulation of nanomaterial behavior *in vivo*. Nanomaterial properties, such as size, shape, material composition, charge, and delivery method (eg, intravenous, topical, oral), determine how a nanoformulation is transported and distributed through the body, and its stability and circulation time.⁷⁰ The effect of size has been particularly well studied. Particles less than 5 nm are rapidly cleared by the renal system, and as size increases, particle filtration and accumulation occur in the cells of the mononuclear phagocyte system in the liver and spleen.^{71,72} It seems evident, when considering these varied factors, that standardized characterization methods are needed. As a result, several studies focused on evaluating the varied physical and chemical properties of nanomaterials have recently been published, using a combination of *in vitro* and *in vivo* techniques.^{73,74} Predictive modeling of accumulation and distribution patterns using computer simulations is proving to be highly effective in conjunction with these analyses. As recently shown, computational models can be designed to incorporate vascular flow and progressive tumor growth over time, effectively serving as a valuable tool in the assessment of nanoparticles before *in vivo* analyses.^{75–77} Further, these tools can be used to incorporate the physical attributes of individual patients, allowing for directed, personalized treatment design.⁷⁸

These modeling efforts must be supported by *in vivo* distribution and efficacy studies. Preclinical evaluation using animal models is standard, but the various factors that must be contemplated in optimizing nanomaterial design (eg, circulation time, immunologic reactivity, vascular permeability) understandably necessitate careful animal selection. To improve translational potential, animal models that mimic the corresponding cancer in humans in terms of affected organ, vasculature, reactivity (immunologically competent), and metastatic advancement should be considered in the selection process, as should study design. To support cancer nanotechnology development, the Nanotechnol-

ogy Characterization Laboratory (ncl.cancer.gov), a federally funded resource, was created to provide the infrastructure and standardized methods needed to assess the translational potential of nanomaterials.⁷⁹ This laboratory, which is available to academic investigators, industry, and government laboratories, conducts preclinical assessments using an established assay cascade, with the ultimate goal of accelerating regulatory review and clinical application. Thus, guidance and support are available to the cancer nanotechnology community to move the field forward. By gaining the ability to control the design and consistent production of nanoplateforms, largely as a result of standardized methods, predictive modeling, and federal efforts, the future of nanotechnology in cancer has significant potential to transform cancer therapeutics and diagnostics.

Conclusions

Nanotechnologies in cancer are expected to dramatically improve treatment and diagnosis. Several laboratories have produced devices and therapeutics mature enough to enter clinical trials, and are mostly in phase I and II trials. Findings of these trials demonstrate reduced side effects and the ability to modify drug dosing, but additional results that strongly support improved survival rates are still needed. The application of novel, on-site in vitro measurement techniques has proven useful in the monitoring and selection of therapies appropriate for a given patient, and may prove to be useful in selecting the most efficacious therapies. Yet, for further progress to occur, the field will require insight from multiple research and medical approaches, not the least of which is input from clinicians, on what is needed by the medical community to better serve the patient. Increasing communication and awareness between nanotechnologists and clinicians is needed to help establish the tumor indications that most require enhanced treatment/diagnosis, and can thereby facilitate the selection and design of the best nanotechnology-based platform. Considerations when submitting nanotherapeutics and diagnostic nanodevices to the FDA have been outlined, but review, as for any other drug submission, is still performed on a case-by-case basis and will vary based on the type and complexity of the platform.⁸⁰ As the process of optimized nanomaterial design continues to evolve, communica-

tion across disciplines, combined with persistence in the novel design and evaluation of nanomaterials using standardized in vivo/in vitro techniques, will undoubtedly accelerate the review of cancer nanotechnologies and their translation into clinical implementation.

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