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Strategic Workshops on Cancer Nanotechnology

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Abstract

Nanotechnology offers the potential for new approaches to detecting, treating and preventing cancer. To determine the current status of the cancer nanotechnology field and the optimal path forward, the National Cancer Institute's Alliance for Nanotechnology in Cancer held three strategic workshops, covering the areas of *in-vitro* diagnostics and prevention, therapy and post-treatment, and *in-vivo* diagnosis and imaging. At each of these meetings, a wide range of experts from academia, industry, the non-profit sector, and the Federal government discussed opportunities in the field of cancer nanotechnology and barriers to its implementation.

Keywords

Nanotechnology; in vitro diagnostics; multi-functional therapeutics; in vivo diagnostics; imaging

Introduction

Cancer is one of the most pressing public health concerns of the 21st century. The statistics are daunting; it was projected that 550,000 people would die of cancer and that another 1.4 million would be diagnosed with the disease in 2009 in the United States alone. Five years ago, the National Cancer Institute (NCI) initiated the NCI Alliance for Nanotechnology (1), in hopes of fostering revolutionary new ways to approach cancer research and care. Nanomaterials have the potential to deliver drugs directly to cancerous tissues and to open up entirely new modalities of cancer therapy. Nanotechnology enhanced microfluidic devices can increase sensitivity and multiplexing capability for cancer marker identification and detection.

The first nanotechnology-based constructs for cancer care are already on the market, including DOXIL® (liposomal doxorubicin) and Abraxane® (albumin bound paclitaxel). Similarly, diagnostic and therapeutic monitoring techniques are benefiting from nanotechnology. New assays utilizing microfluidic based microarrays are being used for genomic and proteomic analysis of cancerous samples while novel nanoparticle-based contrast agents and molecular imaging approaches are entering clinical trials. Most nanotechnology tools currently in development aim to improve diagnostic sensitivity and specificity, or to increase therapeutic index for established chemotherapeutic drugs via selective delivery to cancerous tissue. These tools are advancing cancer research and gradually moving towards the clinic, but are still more

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evolutionary than revolutionary. The identification of new areas of impact and development of novel nanotherapeutics could make the field of cancer nanotechnology more significant and powerful.

To assess the status of the field and provide guidance for future development, the NCI convened three one-day strategic workshops around the following topics:

Workshop I: *In-vitro* Diagnostics and Prevention, February 20, 2008

Workshop II: Therapy and Post-Treatment, March 6, 2008

Workshop III: *In-vivo* Diagnosis and Imaging, March 28, 2008

Participants were asked to consider what the most important goals for cancer research should be for the next 5 or 10 years, how nanotechnology can address these goals and what barriers exist to the integration of nanotechnology and oncology. A summary of discussions from the workshops is presented here.

In-vitro Diagnostics and Prevention Workshop

This workshop hosted presentations by Steven Rosen, M D., (Northwestern University, Evanston, IL) and Gregg Shipp, M D., Ph D., (Nanosphere, Inc., Northbrook, IL) on clinical needs in oncology and David Walt, Ph D., (Yale University, New Haven, CT) and Paul Yager, Ph D., (University of Washington, Seattle, WA) on technological opportunities and challenges. The speakers discussed the limitations of current cancer screening technologies: insufficient sensitivity and specificity to detect precancerous conditions or early stage cancer with a low rate of false positives; inability to determine tumor stage or type; and high cost. They suggested alternative cancer indicators, including constitutive or stimulated proteins, peptides, anomalous cells in fluids or tissues, cell surface markers, genomic or proteomic signatures, and inflammation markers. Ideally, these would distinguish between cancer types and stages and characterize immune response. Technical challenges to the development of these markers include protein heterogeneity, non-specific binding, lack of good capture agents for cells and molecules, and the expense and difficulty of genomic sequencing.

It was agreed that single molecule or cell measurements are stochastic and potentially unrepresentative, making measurement of multiple markers necessary for reliable diagnosis at early stages. Nanotechnology based sensors using quantum dots of multiple colors, Raman probes with distinct spectra, nanoparticle arrays, and nanoscale cantilevers are capable of the high throughput, multiplexed screening this would require. Improvements in genomic sequencing due to nanotechnology were also predicted (2), enabling recognition of cancer specific genes, rapid sequencing of large and heterogeneous samples and in depth profiling of single cells. Enhanced biomarker detection should also result in the discovery and validation of new cancer signatures.

A lively discussion resulted from a suggestion by Dr. Walt that the detection of very early stage disease may be undesirable and lead to unnecessary treatment of lesions that would otherwise be destroyed by the innate immune system. The participants reached a clear consensus in favor of detection at the earliest possible stage.

The speakers also expressed a need for technologies that monitor tumor progression and recurrence, as well as delivery and bioavailability of administered chemotherapeutics and therapeutic efficacy. Dr. Shipp discussed early results from trials of a biobarcode assay that uses oligonucleotide labeled gold nanoparticles to monitor PSA levels in patients following radical prostatectomy; the nanoparticle labeling results in inherent signal amplification. The new assay allows recognition of rising PSA levels as much as two years earlier than standard

ELISA assays (3), so post-surgical disease management can be guided by knowledge of a patient's low or high risk of recurrence.

During working group discussions, participants proposed that highly sensitive and specific multiplexed nano-enabled detection technology would enable speedier marker validation, development of new marker types, e g. cellular metabolic signatures, anoxia, and necrosis, and better characterization of tumor heterogeneity, e g. tumor cell subset, immune cell and cancer stem cell recognition. The working groups also made recommendations for technology development, including:

- Devices for single-cell analysis of circulating tumor cells
- Microfluidics or nanopore based technologies to produce a $1000 genome
- New diagnostics using unprocessed bodily fluids, e g., blood, serum
- Synthetic antibodies with superior affinity, specificity and stability (4)

Participants also raised several technical issues affecting the development of reliable *in vitro* diagnostics: variability in biospecimen collection and preparation procedures, which complicate marker and device validation; poor understanding of molecular recognition processes; and uncertainty regarding how to best characterize disease states using biomarkers – static measurement vs. dynamic tracking of markers, marker concentration vs. absolute number vs. binding affinity measurement, and marker multivalency. Binding to inert surfaces and non-specific binding was considered of particular concern with nanoscale materials, which have large, high energy surfaces.

Therapy and Post-Treatment Workshop

In this workshop, David Parkinson, M D., (Nodality, Inc., San Francisco, CA) and James J. Baker, M D., (University of Michigan, Ann Arbor, MI) presented clinical needs in oncology; Naomi Halas, Ph D, (Rice University, Houston, TX) and Joseph DeSimone, Ph D., (University of North Carolina, Chapel Hill, NC) presented clinical applications of nanotechnology.

Currently, most new anticancer drugs fail in clinical trials, or offer only marginal improvements to the standard of care. Dr. Parkinson suggested that in many of these trials, some patients experienced a strong benefit from the tested drugs, but the trials failed because most patients experienced no effect. This is due to our poor comprehension of the complexity of cancer and interpatient heterogeneity, including variations in cell signaling pathways, tumor microenvironment, and patient metabolism. The key question to be answered is what level of biological characterization is sufficient to handle this heterogeneity and match therapy to patient. There also needs to be a better understanding of the time progression of cancer, if it is to be managed as a chronic disease. Many other drugs fail in trials due to unacceptable toxicity; nanoformulations that enable targeted tumor delivery with a corresponding decrease in side effects could rehabilitate these drugs.

Dr. Halas spoke of the intrinsic therapeutic potential of nanomaterials. Gold nanoshells can convert infrared light into heat at tumor sites, killing cancerous cells, as demonstrated by preclinical studies in the Halas group (5). The FDA has approved clinical trials of this system in head and neck cancers. Plasmonic nanoparticles can also function as contrast agents, raising the possibility of a multi-functional nanoparticle platform combining therapy and monitoring.

However, issues of poor biodistribution and unknown toxicity must be addressed before nanomaterials can be clinically translated. Studies using highly uniform PRINT™ nanoparticles invented at UNC indicate that biodistribution and cellular uptake of nanoparticles depend on the nanoparticle size, shape, deformability and surface chemistry, but the reasons

are poorly understood (6). There is also little understanding of how nanoparticles access the cell interior, complicating efforts to target drugs to intracellular compartments. The role and importance of targeting agents, e g., peptides, oligonucleotides and antibodies, in delivering nanoparticles to cells and tissue must also be understood and compared to mechanistic effects, e g., enhanced retention and permeability of nanomaterials in leaky tumor vasculature.

In discussions following the presentations, participants identified several additional areas in which nanotechnology could impact clinical cancer practices in the next 5 – 10 years. Successful development of the rapid, multiplexed biomarker detection systems discussed in the previous section would lead to more rational and effective tumor stratification and therefore treatment choice. The ability to cheaply collect large data sets of cancer markers across individuals and disease stages should also promote a better understanding of basic cancer biology and heterogeneity, resulting in more predictive models of cancer growth and patient response, including dormancy and metastasis. Personalized treatment regimes could then be constructed by combining the enhanced data collection and cancer models garnered through these detection systems. Highly tumor specific targeting ligands could enhance diagnostic imaging, drug delivery and in vivo monitoring of treatment response. Specific areas recommended for development included:

- Cell surface targeting ligands
- In situ drug release from nanocarriers, triggered externally (e g. plasmonic heating) or chemically (e g. proteolytic peptide cleavage)
- Combination therapies, e g., nanoparticle hyperthermia and drug delivery
- Multifunctional nanoplatforms, e g., liposomes encapsulating imaging agents and drugs or siRNA

Another serious barrier to clinical development discussed is the lack of animal models that simulate human cancers. Most nanotherapeutic delivery exploits tumor structure, making appropriate models indispensible to evaluation of therapeutic efficacy. Unfavorable host immune and cellular response to nanomaterials, the mechanisms of which are poorly understood, must also be resolved before nanomaterials can be clinically useful.

In-vivo Diagnosis and Imaging Workshop

In this workshop, James Olson, M D., Ph D., (Fred Hutchinson Cancer Research Center, Seattle, WA) Shimon Weiss, Ph D., (University of California, Los Angeles, CA) and Renata Pasqualini, Ph D., (University of Texas M. D. Anderson Cancer Center, Houston, TX) presented wish lists of desired cancer imaging capabilities, surveyed promising nanotechnologies for imaging applications and outlined the gaps between technology development and clinical application. Oncologists wish had they sufficient spatial resolution and molecular recognition abilities to detect very early stage tumors, diagnose tumor type, and define metastases without surgery or pathology. This detailed imaging information could then be used to guide tumor and lymph node removal using intra-operative imaging or to track drug delivery and response in disease sites. This information could also be used to identify emerging resistance to chemotherapy. Dr. Olson suggested that amplification of signal from rare events, such as mitosis and anaplasia, could be used to differentiate diseased from healthy tissue, and that recognition of cellular differences, such as open versus closed chromatin, could be used to distinguish tumor types. Other diagnostically useful measures suggested were differences in the ratio of the nucleus to the cytoplasm in suspected tumors, differences in oxygen tension, pH differences, heterogeneity, and specific tumor markers.

Participants recognized the opportunity afforded by nanotechnology to perform rapid, multiplexed molecular imaging in multiple modalities. If nanoprobes targeted to multiple

markers could accumulate at the tumor and be detected using MRI, PET and/or near-infrared imaging, the information could be combined to non-invasively diagnose tumor type and stage. Additional targeting to recognize multiple cell types (e g., healthy, tumor, stem) or activities could give a complex picture of tumor microenvironment and metabolism and track tumor growth and therapeutic response. Nanoparticles coated with enzymatically or pH sensitive peptides that experience aggregation, fluorescent quenching or some other measureable signal change in response to peptide activation by tumor cells are already being explored for this type of imaging. Dr. Weiss suggested combining these probes with implantable devices, themselves recognizable by MRI, that conduct detailed molecular analyses of tumor tissue before, during and after therapy (7). Deep tissue imaging can also be achieved using fiber optics to access and illuminate targets that have previously been targeted with optically active nanomaterials, e g., quantum dots or Raman spectroscopy tags.

One promising avenue of research discussed by Dr. Pasqualini is the development of organ specific and angiogensis related vascular ZIP codes (8) that enable region or activity based targeting strategies. This technique can be used to map molecular diversity and target accessible tumor receptors that can internalize and accumulate nanoparticles, increasing image signal. As an example, she described a bioinorganic nanoparticle that binds to a lung vascular endothelial receptor and that may provide a predictive tool for drug response based on imaging data (9).

To attain these ambitious goals, workshop participants identified several necessary developments. Existing imaging strategies must be quantified so that findings across centers can be compared and the effects of nanotechnology on imaging capabilities measured. A reasonable number of targets for imaging must be determined for development, as well as measures of therapeutic efficacy in addition to apoptosis, and automated image analysis software will be necessary to make meaningful sense of the data collected. There is also a pressing need for a battery of in vitro and in vivo tests to develop go/no go criteria for nanoparticles for in vivo use. Toxicity and targeting efficacy standards must be established. During discussions, participants also identified several specific technologies as being ready for development, including:

- Automated, microfluidics-based imaging probe synthesis (10)
- Carbon nanotube based x-ray imaging devices/CT scanners
- Nanomaterials with increased relaxivities for MR-based imaging
- Nanoparticle contrast agents with external activation of therapeutic effect
- Substitution of a PET-suitable isotope into an approved nanoparticle-based therapeutic for biodistribution studies
- A national GMP manufacturing facility for scaling up nanoparticle production

These applications require development of suitable cancer biomarkers and targeting ligands, as well as tools to monitor and evaluate nanomaterial pharmacokinetics and cellular interactions in vivo. Studies are also required to determine the lower limits of tumor size detectable using in vivo imaging.

Summary

Workshop participants believed that nanotechnology applied to clinical oncology practice has the potential to better monitor therapeutic efficacy, provide novel methods for detecting and profiling early stage cancers, and enable surgeons to delineate tumor margins and sentinel lymph nodes. The field is well positioned to provide improved methods for imaging and staging cancers and to more effectively deliver therapeutics in a targeted manner to tumors. However, nanotechnology based cancer therapies and diagnostics needs to pass several critical tests

before the future of the field is assured. These include successful in vivo delivery of a targeted therapeutic, establishing viability of both targeting chemistry and nanomaterial pharmacokinetics, and deployment of a multiplexed in vitro diagnostic for cancer, establishing biomarker and capture agent validity and device design integrity.

Ultimately, if nanotechnology researchers can establish methods to detect tumors at a very early stage, prior to vascularization and metastasis, cancer will become a disease amenable to complete cure via surgical resection. The impact on the disease survival rates and disease management expenditures could be exceedingly high.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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