CANCER DIAGNOSIS AND THERAPEUTICS: NEWER PROMISING AVENUES OFFERED BY NANOTECHNOLOGY

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Summary

Nanotechnology is multidisciplinary field that involves the design and engineering of objects <500 nanometers in size. As far as pharmaceutical sector is concerned, in the last several decades, nanotechnology has been studied and developed primarily for use in novel drug-delivery systems; e.g. liposomes, gelatin nanoparticles, micelles. Nanotechnology offers an extraordinary, paradigm-changing opportunity to make significant advances in cancer diagnosis and treatment. Current imaging techniques like computed tomography, magnetic resonance imaging, positron emission tomography suffer from a common shortcoming — they just aren't sensitive enough to accurately find the smallest tumors that are most easily and effectively treated. Nanotechnology may be able to provide that leap in sensitivity that would not only impact today's approach to therapy but could lead to entirely new pathways for both detecting and treating cancer. A recent explosion in engineering and technology has led to the development of many new nanoscale platforms, including nanovectors, nanowires, nanocantilever arrays, quantum dots, nanoshells, gold nanoparticles, paramagnetic nanoparticles, carbon nanotubes and near-infrared fluorescence nanoparticles that have emerging implications in cancer diagnostics and therapeutics.

Keywords: Nanoparticles, Quantum dots, Nanocantilever arrays, Nanowires, Nanopores, Carbon Nanotubes, Liposomes, Dendrimers, Gold Nanoshells

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Introduction

Nanotechnology is multidisciplinary field that involves the design and engineering of objects <500 nanometers [nm] in size, more specifically 1-100nm. Nanotechnology offers an extraordinary, paradigm-changing opportunity to make significant advances in cancer diagnosis and treatment. The past quarter century of outstanding progress in fundamental cancer biology has not translated into even distantly comparable advances in the clinic. Inadequacies in the ability to administer therapeutic moieties so that they will selectively reach the desired targets with marginal or no collateral damage has largely accounted for the discrepancy [1]. Most striking is the recognition that only between 1 and 10 parts per 100,000 of intravenously administered monoclonal antibodies reach their parenchymal targets *in vivo* [2]. Similar limitations apply to contrast agents for imaging applications. There are two general, synergistic goals that should be striven for to increase the efficacy per dose of any therapeutic or imaging contrast formulation: to increase its targeting selectivity [3] and to endow the agent[s] comprising the therapeutic formulation with the means to overcome the biological barriers that prevent it from reaching its target [4]. The hypothesis offered is that nanotechnology, if properly integrated with established cancer research, provides extraordinary opportunities to meet these challenges. It has the potential to offer solutions to the current obstacles in cancer therapies, because of its unique size [1-100nm] and large surface-to-volume ratios [5].

Nanotechnology In Cancer Diagnosis

Current imaging methods like computed tomography [CT] imaging, magnetic resonance imaging [MRI], positron emission tomography [PET] are restricted in spectrum range, penetration depth, cell targeting, and signal/noise clarity [6]. Also, most imaging methods produce static images, snapshots of a tumor at one particular time that do not reveal much about dynamic events, such as the binding of a drug to a particular tissue. But increasingly, it appears that nanotechnology may be able to provide that leap in sensitivity that would not only impact today's approach to therapy but could lead to entirely new pathways for both detecting and treating cancer. In July 2006, an article by Sathe et al. demonstrated the use of nanotechnology in cancer detection [7]. The potential for nanostructures to enter and analyze single cells suggests they could detect molecular changes even when they occur only in a small percentage of cells.

Quantum Dots

Are nanosized semiconductor particles, made of cadmium selenide [CdSe], cadmium sulfide [CdS] or cadmium telluride [CdTe] with an inert polymer coating. The semiconductor material used for the core is chosen based upon the emission wavelength range being targeted: CdS for UV-blue, CdSe for the bulk of the visible spectrum, CdTe for the far red and near-infrared. They glow when they are stimulated by light of particular wavelength. The color of the light depends on the size of the crystal. To detect cancer, beads containing quantum dots can be designed to bind to sequences of DNA that are associated with cancer. When the quantum dots are stimulated with light, they will emit their unique bar codes, or labels, making the critical, cancer-associated DNA sequences visible. Dual-functioning beads comprised of quantum dots and iron oxide nanocrystals embedded in silica beads having high imaging qualities have also been developed [7].

Figure 1: Cadmium selenide [CdSe] Quantum Dots

Nanoscale Cantilevers

Biomolecular sensors with the ability to 'multiplex' massively — that is, to detect a large number of different molecular species at the same time — are being developed for serum and tissue proteomics-based cancer diagnostics, prognostics and therapeutic-efficacy monitoring. Promising emerging approaches to multimolecular sensing include mechanical sensors such as microcantilever and nanocantilever arrays [8-10]. These comprise a large number of beams that deflect when altered DNA sequences or proteins that are present in certain types of cancer bind. They can detect extremely small forces, stresses and masses. The deflections are either observed directly by laser light or generate detectable shifts in the physical properties of the beam, such as their resonant-vibration frequency. They are capable of sensing the presence of single molecules of clinical importance. This will be useful in detecting early molecular events in the development of cancer. Microcantilever-based, multiplexed DNA assays to detect *BRCA1* mutations were recently introduced [11].

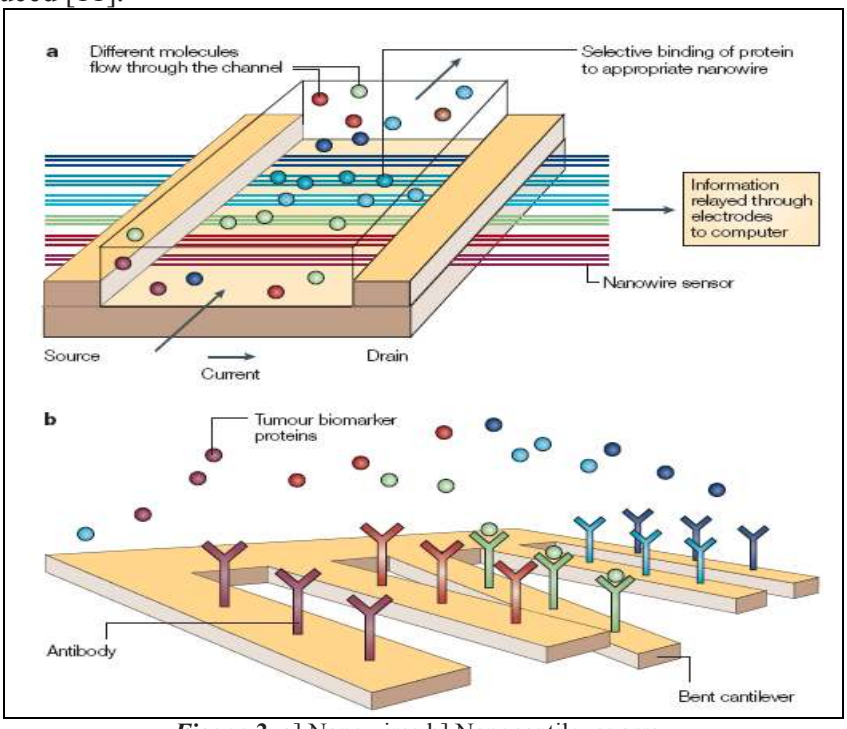

Figure 2: a] Nanowires b] Nanocantilever array

Nanowires

Nanoscale sensing wires made of metal atoms, silicon, or other materials that conduct electricity, can be coated with antibodies that will bind to tumor cells. They operate as nanoscale field-effect biotransistors; incredibly sensitive to such binding events and respond by altering the electrical current flowing through them, transmit their information through electrodes to computers and thus can form the basis of ultra sensitive molecular detectors [12, 13].

Nanoparticle-based Contrast Imaging Agents

Several types of nanoparticle for the enhancement of MRI contrast have been used clinically and in research protocols. These include gadolinium-based [14], iron oxide- based nanoparticles [15-20] and multiple-mode imaging contrast nano-agents that combine magnetic resonance with biological targeting [21] and optical detection [21, 22]. Low-density lipid nanoparticles have been used to enhance ultrasound imaging [23, 24]. In 2003 Weissleder and colleagues [25] recently demonstrated that lymphotropic paramagnetic nanoparticles allow the MRI imaging of clinically occult lymph-node metastases in patients with prostate cancer, which are not detectable by any other non-invasive approach. In July 2006, an article by Sathe et al. described development of dual-functioning beads comprised of quantum dots and iron oxide nanocrystals embedded in silica beads. These particles were able to target specific cells, due to the iron oxide crystals, and have high imaging qualities, due to the quantum dot component [7]. Nanoparticles are also showing promise for the *ex vivo* detection of biomarkers. For instance, fluorophore- laden silica beads have been used for the optical identification of leukaemia cells in blood samples [26].

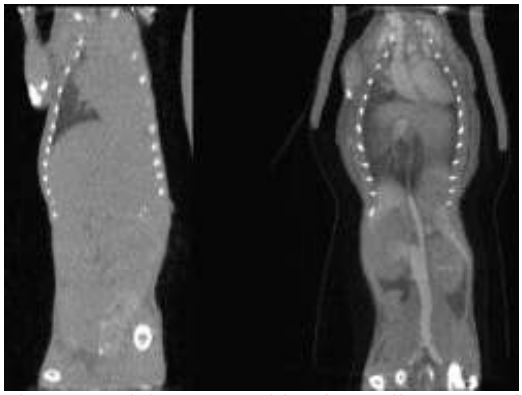

Figure 3: Polymer-coated bismuth nanoparticles are capable of revealing anatomical details that are invisible on a standard CT image. The CT image on the left comes from a live mouse before injection of the bismuth nanoparticles, while the CT image on the right clearly delineates the vasculature, heart and other organs. *[Courtesy: Ralph Weissleder, M.D., Ph.D., MIT-Harvard Center of Cancer Nanotechnology Excellence]*

Nanopores

As DNA passes through a nanopore, the shape and electrical properties of each base on the strand can be monitored. Because these properties are unique for each of the four bases that make up the genetic code, the passage of DNA through a nanopore can be used to decipher the encoded information, including errors in the code known to be associated with cancer. [27,28,29]

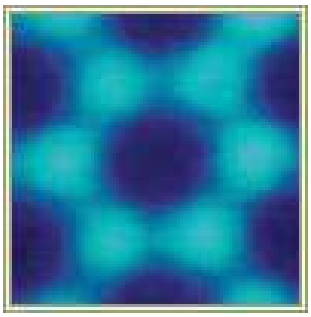

Figure 4: Nanopore

Carbon Nanotubes

Even smaller than nanopores are nanotubes. Nanotubes, hollow carbon rods [30,31] about half the diameter of a molecule of DNA, will also help identify DNA changes associated with cancer. Beyond detecting the presence of altered genes, these materials may help to pinpoint the exact location of those changes. Mutated regions associated with cancer are first tagged with bulky molecules. Using a nanotube tip resembling the needle on a record player, physical shape of DNA can be traced [32]. A computer translates this information into a topographical map. The bulky molecules identify the regions on the map where mutations are present. Since the location of mutations can influence the effects they have on a cell, these techniques will be important in predicting development of cancerous growth.

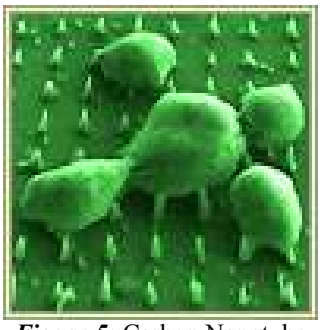

Figure 5: Carbon Nanotube

Nanotechnology in cancer therapeutics

Nanotechnology may also be useful for eradicating cancer cells without harming healthy, neighboring cells by creating therapeutic agents that target specific cells and deliver the toxin in a controlled, time-release manner. Efforts are being made to create single agents that are able to both detect cancer and deliver treatment. The ultimate goal is to develop nanoparticles that will circulate through the body, detect cancer-associated molecular changes, assist with imaging, release a therapeutic agent, and then monitor the effectiveness of the intervention.

Liposomes

Liposomes are the archetypal, simplest form of a nanovector. They use the overexpression of fenestrations in cancer neovasculature to increase drug concentration at tumor sites. Liposome-encapsulated formulations of doxorubicin were approved 13 years ago for the treatment of Kaposi's sarcoma, and are now used against breast cancer and refractory ovarian cancer. Liposomes continue to be refined and applied to more cancer indications [3,33,34]. They are only the first in an ever-growing number of nanovectors under development for novel, more efficacious drug-delivery modalities [1,35].

Dendrimers

A dendrimer is a tree-like highly branched polymer molecule [Greek *dendra = tree*]. Dendrimers are synthesized from monomers with new branches added in discrete steps ["generation"] to form a tree-like architecture [36]. A useful feature of dendrimers is their branching shape, which gives them vast amounts of surface area to which therapeutic agents or other biologically active molecules can be attached. A single dendrimer can carry a molecule that recognizes cancer cells, a therapeutic agent to kill those cells, and a molecule that recognizes the signals of cell death.

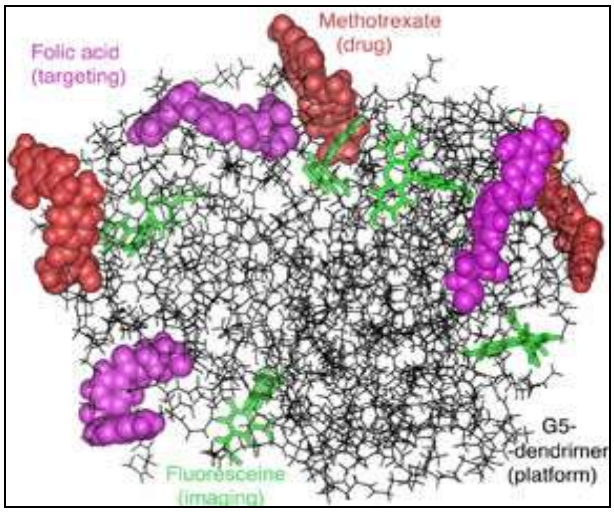

Figure 7: A Computer model of dendrimer [*Citation: Cancer Research 2005: 65 (1), June 15, 2005]*

Gold Nanoshells

Nanoshells are a novel class of optically tunable nanoparticles that consist of a dielectric core surrounded by a thin gold shell [37]. Based on the relative dimensions of the shell thickness and core radius, nanoshells may be designed to scatter and/or absorb light over a broad spectral range including the near-infrared [NIR], a wavelength region that provides maximal penetration of light through tissue [38]. The ability to control both wavelength-dependent scattering and absorption of nanoshells offers the opportunity to design nanoshells which provide, in a single nanoparticle, both diagnostic and therapeutic capabilities. The absorption of light by the nanoshells creates an intense heat that is lethal to cells [39,40]. Antibodies that recognize cancer cells can be linked to nanoshells so as to achieve tumor targeting, e.g. anti-HER2 nanoshells to

detect and destroy breast carcinoma cells that overexpress HER2, a clinically relevant cancer biomarker [41]. Although the gold surfaces of nanoshells are generally considered to be biocompatible, stealthing polymers such as poly[ethylene glycol] [PEG] may be attached to nanoshells surfaces to further enhance biocompatibility and improve blood circulation times [42].

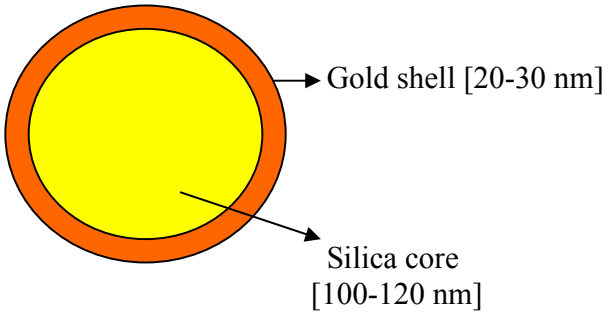

Figure 8: Schematic figure of a Gold Nanoshell

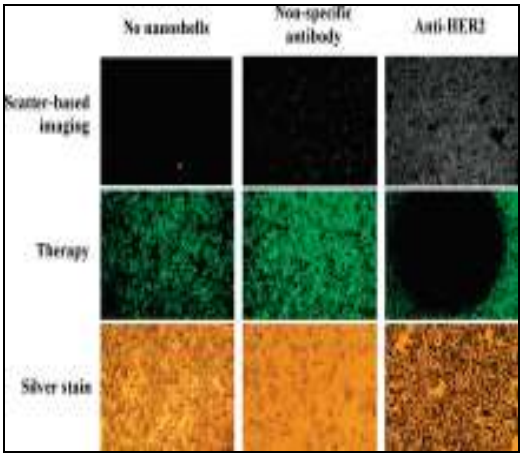

Figure 11: Increased contrast [top row, right column] and cytotoxicity [dark spot] in cells treated with a NIR emitting laser following nanoshell exposure [middle row, right column] compared to controls [left and middle columns]. [*41*]

Nanoparticles

Examples include nanoparticles of poly[ethylene glycol] [PEG] surface modified thiolated gelatin [43], poly[ethylene oxide]-modified poly[beta-amino ester] [PEO-PbAE] [44] as well as nanoparticle-aptamer bioconjugates [45]. Administration of paclitaxel through PEO-PbAE nanoparticles in mice, bearing human ovarian adenocarcinoma xenograft resulted in more efficient in drug delivery, reduced toxicity and decreased tumor growth rates in comparison to the paclitaxel aqueous solution [44]. The use of docetaxel-encapsulated nanoparticle-aptamer bioconjugates demonstrated a decrease in tumor size from approximately 300 mm^3 to 120 mm^3 [45,46,47]]. *Abraxane*® approved by USFDA in January 2005 for the treatment of metastatic

breast cancer, consists of paclitaxel nanoparticles that are conjugated to albumin molecules. By using targeted nanoparticles, researchers at California Institute of Technology have demonstrated that systemically delivered siRNA can slow the growth of tumors in mice without eliciting the toxicities often associated with cancer therapies.

Clinical Trials

In April 2004, the US based nanotechnology company pSivida, announced the very promising results of the Phase 2 clinical trials, undertaken at the Singapore General Hospital, of its product "BrachySil" for patients with liver cancer. The same product is in phase II for pancreatic cancer. Insert Therapeutics in collaboration with California Institute of Technology has completed phase I trials of targeted nanoparticles in 2007, and phase II trials are about to begin. Phase I trials of biocompatible magnetic iron oxide nanoparticles for recurrent prostate cancer have also been completed in 2007 at Humboldt University Hospital Cherité, Berlin, Germany. Nanospectra Biosciences, based in Houston, is in the process of getting FDA approval for clinical trials of gold nanoshells [*AuroShell*™] developed by Rice University, for treating head and neck cancer [*AuroLase*™ Cancer Therapy].

Cancer Nanotechnology: The Challenges

Some of the principal challenges in application of nanotechnology in the oncology field are discussed below.

Targeted delivery of therapeutic or imaging agents

Multiple targeting strategies might be used to preferentially concentrate injected agents at tumor sites. For Instance quantum dot bioconjugates with targeting antibodies have been used to recognize molecular signatures including ERBB2. Several antigens have been used to preferentially direct nanoparticles to angiogenic endothelium. For example, targeting $\alpha_v\beta_3$-integrin, which is found on endothelial cells, was used with perfluorocarbon- based nano-emulsions for the MRI imaging of neovasculature [48,49] and anti-angiogenesis therapy in murine models of melanoma and colon adenocarcinoma [50,51]. Epidermal growth factor [EGF] receptor was proposed to target EGF-derivatized silicon particulates carrying the pore-forming protein melittin to provide selective action to lyse the membranes of cells in angiogenic endothelium [52, 53]. The peptide mediated nuclear targeting of gold nanoparticles was reported [54]. Another class of targeting methods uses external energy as a trigger for the localized activation of cytotoxic action, and has been demonstrated in animal models. Examples are the use of focused ultrasound to burst lipid encapsulated 'microbubbles' [55]; photodynamic therapy on silica-based carriers [56,57]; and the localized thermal ablation of cancer lesions by the combined use of gold nanoshells and optical activation in the near-infrared region, by which deep tissue penetration can be achieved [58,59,60].

Engineering nanoparticles to circumvent biological and biophysical barriers

Biological barriers might arise in the form of tight junctions between epithelial cells, as is the case for the blood–brain barrier [BBB], which impedes the extravasation of vascularly

injected agents. Nanotechnology-based systems have shown efficacy in crossing the BBB by virtue of the properties of their constituent core materials [61, 62–65]. Endothelial vascular permeability might be increased by the co-administration of a bradykinin antagonist [66]. The co-localized delivery of permeation enhancers such as zonula-occludens toxin, which reversibly opens tight junctions, affords the penetration of orally administered biomolecular agents through the intestinal epithelium, which is a very effective barrier, into the vascular compartment [67,68]

Toxicity profile

For example, most of the nanovectors used in high contrast imaging [such as quantum dots] are toxic [69] and cannot be applied to the body. Over long periods of time, nanoparticles may aggregate, potentially blocking arteries and veins or even blocking the kidneys, and thereby creating a host of new problems. For any nanovector to be successful in clinical application, it must be either completely destroyed or biodegradable *in vivo*. Secondly, nanovectors might also trigger sensitization reactions. For example, antibodies specific to fullerenes have been described; dendrimers and protein-dendrimer conjugates have shown strong immunogenic response in these studies [70]. Therefore, counter measures to suppress such reactions for killing cancer cells must be devised.

Regulatory issues and FDA approval

However promising nanovector delivery systems might be, the enthusiasm for them must be placed in the context of stringent regulatory approval perspectives. The relevant issues go well beyond considerations of biocompatibility of the carriers [69], their biodistribution [71] and the reliability of their production protocols [72], which of course remain central concerns. By their very tripartite nature, nanoparticles arguably fall under the purview of the three branches of regulatory agencies such as the Food and Drug Administration [FDA]: drugs, medical devices and biological agents. Therefore, they might have to be examined from these three perspectives accordingly [73].

Conclusions

Human body is built upon a foundation of nature-made nanostructures – genes, proteins, cells – that may be best approached on their own scale. The potential for nanostructures to enter and analyze single cells suggests they could detect molecular changes even when they occur only in a small percentage of cells. Efforts are being made to develop nanoparticles that will circulate through the body, detect cancer-associated molecular changes, assist with imaging, release a therapeutic agent, and then monitor the effectiveness of the intervention. In nutshell, nanotechnology with its power of 10^{-9} has the potential to offer solutions to the current obstacles in cancer therapies, because of its unique size and large surface-to-volume ratios. In the not too distant future, dozens of intriguing nanodevices shall transform cancer diagnosis & treatment. Thus ***little nano, the next big thing,*** is opening up potential newer avenues in the field of oncology

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