Nanotechnology Applications in Cancer

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Abstract

Cancer nanotechnology is an interdisciplinary area of research in science, engineering, and medicine with broad applications for molecular imaging, molecular diagnosis, and targeted therapy. The basic rationale is that nanometer-sized particles, such as semiconductor quantum dots and iron oxide nanocrystals, have optical, magnetic, or structural properties that are not available from molecules or bulk solids. When linked with tumor targeting ligands such as monoclonal antibodies, peptides, or small molecules, these nanoparticles can be used to target tumor antigens (biomarkers) as well as tumor vasculatures with high affinity and specificity. In the mesoscopic size range of 5–100 nm diameter, nanoparticles also have large surface areas and functional groups for conjugating to multiple diagnostic (e.g., optical, radioisotopic, or magnetic) and therapeutic (e.g., anticancer) agents. Recent advances have led to bioaffinity nanoparticle probes for molecular and cellular imaging, targeted nanoparticle drugs for cancer therapy, and integrated nanodevices for early cancer detection and screening. These developments raise exciting opportunities for personalized oncology in which genetic and protein biomarkers are used to diagnose and treat cancer based on the molecular profiles of individual patients.

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INTRODUCTION

The Cancer Problem

Human cancer is a complex disease caused by genetic instability and accumulation of multiple molecular alterations (1, 2). Current diagnostic and prognostic classifications do not reflect the whole clinical heterogeneity of tumors and are insufficient to make predictions for successful treatment and patient outcome (3, 4). Most current anticancer agents do not greatly differentiate between cancerous and normal cells, leading to systemic toxicity and adverse effects. In addition, cancer is often diagnosed and treated too late, when the cancer cells have already invaded and metastasized into other parts of the body. At the time of clinical presentation, for example, more than 60% of patients with breast, lung, colon, prostate, and ovarian cancer have hidden or overt metastatic colonies (5). At this stage, therapeutic modalities are limited in their effectiveness. Due to these problems, cancer has overtaken heart disease as the leading cause of death for adults in the United States [United States Cancer Statisics, Centers for Disease Control and Prevention (CDC) **http://www.cdc.gov/cancer/npcr/uscs**].

Current problems and unmet needs in translational oncology include (*a*) advanced technologies for tumor imaging and early detection, (*b*) new methods for accurate diagnosis and prognosis, (*c*) strategies to overcome the toxicity and adverse side effects of chemotherapy drugs, and (*d*) basic discovery in cancer biology leading to new knowledge for treating aggressive and lethal cancer phenotypes such as bone metastasis. Advances in these areas will form the major cornerstones for a future medical practice of personalized oncology in which cancer detection, diagnosis, and therapy are tailored to each individual's tumor molecular profile and also for predictive oncology in which genetic/molecular markers are used to predict disease development, progression, and clinical outcomes.

Cancer Nanotechnology

Cancer nanotechnology is emerging as a new field of interdisciplinary research, cutting across the disciplines of biology, chemistry, engineering, and medicine, and is expected to lead to major advances in cancer detection, diagnosis, and treatment (6, 7). The basic rationale is that metal, semiconductor, and polymeric particles have novel optical, electronic, magnetic, and structural properties that are often not available from individual molecules or bulk solids (8–10). Recent research has developed functional nanoparticles that are covalently linked to biological molecules such as peptides, proteins, nucleic acids, or small-molecule ligands (11–18). Medical applications have also appeared, such as the use of superparamagnetic iron oxide nanoparticles as a contrast agent for lymph node prostate cancer detection (19) and the use of polymeric nanoparticles for targeted gene delivery to tumor vasculatures (20). New technologies using metal and semiconductor nanoparticles are also under intense development for molecular profiling studies and multiplexed biological assays (21–25).

Cancer Biomarkers

Biomolecular markers or biomarkers include altered or mutant genes, RNAs, proteins, lipids, carbohydrates, and small metabolite molecules, and their altered expressions that are correlated with a biological behavior or a clinical outcome. Most cancer biomarkers are discovered by molecular profiling studies based on an association or correlation between a molecular signature and cancer behavior. In the cases of both breast and prostate cancer, a deadly step is the appearance of so-called lethal phenotypes, such as bone-metastatic, hormone-independent, and radiation- and chemotherapy-resistant phenotypes. It has been hypothesized that each of these aggressive behaviors or phenotypes could be understood and predicted by a defining set of biomarkers. By critically defining the interrelationships among these biomarkers, it could be possible to diagnose and prognosticate cancer based on a patient's molecular profile, leading to personalized and predictive medicine. That is, a unique molecular profile can be used to predict the tumor's invasive and metastatic potential, its ability to survive and grow under androgen-deprived and hypoxia and metabolic stress conditions, and the potential of certain cancer cells to evade host immune surveillance.

Prognosis: prediction of how a patient's disease will progress and its clinical outcome

Superparamagnetic: often associated with single-domain iron nanoparticles that become ferromagnetic in the presence of an external magnetic field but lose magnetization when the magnetic field is removed

Biomarkers: any biomolecules or analytical features associated with a disease or its behavior

One of the first molecular profiling studies was reported by Golub et al. (26) who showed that gene expression patterns could classify tumors, yielding new insights into tumor pathology such as stage, grade, clinical course, and response to treatment. Gene expression studies of cell lines further revealed that the molecular signature of each tumor is a result of the combined tumoral, stromal, and inflammatory factors of the original heterogeneous tumor (27). The first clinical correlation of gene expression patterns with clinical outcome was reported for diffuse large B-cell lymphoma (28), a clinically heterogeneous disease. Whereas most (60%) of the patients succumbed to the disease, the remainder responded well to therapy and had prolonged survival. This variability in disease progression was correlated with a distinct pattern of gene expression. The concept of a specific molecular portrait for each patient's tumor was later validated by Perou et al. (29) and Bittner et al. (30).

Most recent work on cancer molecular profiling by Rubin, Chinnaiyan, and their coworkers has combined cDNA microarrays with tissue microarrays for biomarker discovery and immunohistochemical validation (31–38). For prostate cancer, a number of gene and protein biomarkers have been identified, including p504S (α-methylacyl coenzyme A racemase or AMAC, an enzyme involved β-oxidation of fatty acids), hepsin (HPN, a transmembrane serine protease), Pim-1, protease/KLK4, prostein, EHZ 2, and STEAP (39, 40). These markers appear to be excellent indicators of aggressive cancer behavior, such as metastasis and androgen independence.

Personalized Oncology

For applications in individualized therapy, biomarkers enable the characterization of patient populations and quantification of the extent to which new drugs reach their intended targets (41, 42). One example is the drug trastuzumab (Herceptin, Genentech/Roche), a monoclonal antibody designed to target amplified and overexpressed *ERBB2* (also known as HER2) tyrosine kinase receptor found in ∼25%–30% of breast cancers. FDA approval of trastuzumab was predicated on the availability of a test to detect *ERBB2* overexpression. Both an immunohistochemistry assay for the expressed protein (HercepTest, Dako) and a nucleic acid–based fluorescence in situ hybridization (FISH) test (PathVysion, Abbott) have been approved as in vitro diagnostics to guide trastuzumab treatment decisions. In another example, the clinical response of lung cancer patients to the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor gefitinib (Iressa[TM], AstraZeneca) is associated with a small number of genetic mutations (43, 44). Thus, a molecular diagnostic test could be used to identify patients that are most likely to respond to this drug.

Despite these advances, critical studies that can clearly link biomarkers with cancer behavior remain a significant challenge. One difficulty is that most cancer tumors (especially prostate and breast cancer) are highly heterogeneous, containing a mixture of benign, cancerous, and stromal cells. Current technologies for molecular profiling, including RT-PCR, gene chips, protein chips, two-dimensional (2-D) gel electrophoresis, and biomolecular mass spectrometry (e.g., MALDI-MS, ES-MS, and SELDI-MS), are not designed to handle this type of heterogeneous sample (45, 46). Furthermore, a limitation shared by all these technologies is that they require

Nanotechnology

Informatics

Detection

Diagnostics

Therapeutics
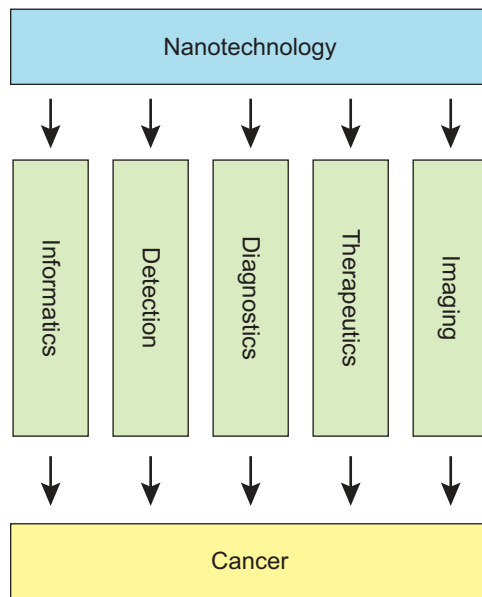
Imaging

Cancer

Figure 1

Schematic diagram showing nanotechnology applications in cancer through molecular tumor imaging, early detection, molecular diagnosis, targeted therapy, and cancer bioinformatics.

destructive preparation of cells or tissue specimens into a homogeneous solution, leading to a loss of valuable 3-D cellular and tissue morphological information associated with the original tumor. The development of nanotechnology, especially bioconjugated nanoparticles, provides an essential link by which biomarkers could be functionally correlated with cancer behavior. **Figure 1** illustrates nanotechnology applications in cancer through molecular imaging, diagnosis, early detection, targeted therapy, and cancer bioinformatics. In the following, we describe the design and development of nanoparticle probes and their applications in cancer.

NANOPARTICLE PROBES

A prototype nanoparticle is semiconductor quantum dots (QDs), tiny light-emitting particles on the nanometer scale that are emerging as a new class of fluorescent probes for in vivo biomolecular and cellular imaging (11–18) (**Figure 2**). In comparison with organic dyes and fluorescent proteins, QDs have unique optical and electronic properties. QDs have molar extinction coefficients that are 10–50 times larger than that of organic dyes, which make them much brighter in photon-limited in vivo conditions. Further, QDs emission wavelengths are size-tunable. For example, CdSe/Zns QDs of approximately 2 nm in diameter produce a blue emission, whereas QDs approximately 7 nm in diameter emit red light (47). In recent work, researchers have pushed the emission wavelength into the near infrared (650 nm to 950 nm), to take advantage of improved tissue penetration depth and reduced background fluorescence at these wavelengths (48). A key property for in vivo imaging is the broad QD Stokes shift, which can be as large as 300–400 nm, depending on the wavelength of the excitation

Quantum dots (QDs): tiny particles on the nanometer scale with quantum-confinement properties such as size-tunable light emission, most often made of semiconductors such as CdSe

Figure 2

Semiconductor quantum dots with quantum confinement and size-tunable optical properties. This image shows ten distinguishable emission colors of ZnS-capped CdSe quantum dots excited with a near-UV lamp. From left to right (*blue to red*), the emission maxima are located at 443, 473, 481, 500, 518, 543, 565, 587, 610, and 655 nm.

light (49). In conjunction with broadband absorption and narrow emission peaks of QDs, this property allows multiplexed imaging applications in which one light source is used to simultaneously excite multicolor QDs without the need for complicated instrumentation. Another important feature is the long-term photostability of QD imaging probes, which opens the possibility of investigating the dynamics of cellular processes over time, such as continuously tracking cell migration, differentiation, and metastasis. These properties have made QDs a topic of intensive interest in cancer biology, molecular imaging, and molecular profiling.

Dual-Modality Probes

Optical imaging is highly sensitive, but its applications in vivo and in human are hampered by a limited penetration depth in tissue and the lack of anatomic resolution and spatial information. Although near-infrared wavelengths can be used to improve the penetration depth, and 3-D fluorescence tomography can be used to provide spatial information (50, 51), other imaging modalities, such as magnetic resonance imaging (MRI), are much better for tomography and 3-D imaging. Thus, there has been considerable interest in developing dual-modality contrast agents for combined optical and MRI, which has exceptional tissue contrast and spatial resolution and has been widely used in the clinical setting. For example, by reacting superparamagnetic iron oxide nanoparticles with the fluorescent dye Cy5.5, Josephson and coworkers (52) have developed dual magneto-optical probes that are able to bind to apoptotic cells and are detectable by both fluorescence and MRI. Similarly, dual magnetic and optical imaging probes have been used to yield highly detailed anatomic and molecular information in living organisms (53). These probes are prepared by conjugation of peptides to cross-linked iron oxide amine (amino-CLIO), either by a disulfide linkage or a thioether linker, followed by the attachment of the dye Cy5 or Cy7. Fluorescence quenching of the attached fluorochrome occurs by interaction with the iron oxide

Magnetic resonance imaging (MRI): a medical imaging modality that measures proton spin relaxations (T1 and T2) in primarily water molecules for 3-D reconstruction of soft tissues such as the brain

core, and also by electronic coupling among the dye chromophores (self-quenching). This class of dual-modality probes provides the basis for "smart" nanoparticles, capable of pinpointing their position through their magnetic properties, while providing information on their environment by optical imaging.

Recent research has shown that QDs can be linked with Fe_2O_3 and FePt to generate dual-function nanoparticles (54, 55). Others have entrapped Gd on the QD surface using polymer-conjugated lipids to form dual-modality probes, but it is not clear whether these types of "hetero" nanostructures would be useful for in vivo medical imaging (56, 57). Research in our own group has created a new class of dual-modality nanoparticles by attaching a cluster of paramagnetic gadolinium chelates to polymer-coated QDs. Preliminary cellular and in vivo animal studies demonstrated that this class of nanoparticle is biocompatible and detectable by both fluorescence and MRI. In comparison with previous work, the polymer-protected QDs offer excellent optical properties (high-fluorescence quantum yields, narrow spectral widths, and high photostability), and the attached Gd chelates lead to significant T1 contrast enhancement with a brightening effect in MRI, as opposed to the T2 contrast with a darkening effect offered by iron oxide–based contrast. By linking to targeting ligands through a biocompatible polyethylene glycol (PEG) spacer, these dual-modality nanoparticle probes are promising for in vivo tumor imaging in animal models.

Multifunctional Platforms

Nanoparticles also offer a wide range of surface functional groups allowing chemical conjugation to multiple diagnostic and therapeutic agents. It is thus possible to design and develop multifunctional nanostructures that could be used for simultaneous tumor imaging and treatment, a major goal in cancer research and development. However, progress has been slow, and promising multifunctional platforms, such as dendrimers, liposomes, and PEBBLES (probes encapsulated in biologically localized embedding), have not been able to deliver diagnostic and therapeutic agents to tumors in a selective and efficient manner (58–62). Most of these studies are still at an early or proof-of-concept stage using cultured cancer cells, which are not immediately relevant to in vivo imaging and treatment of solid tumors.

MOLECULAR CANCER IMAGING

In comparison with traditional in vivo imaging probes or contrast agents [such as radioactive small molecules in positron emission tomography (PET) and single photon emission computed tomography (SPECT), gadolinium compounds in MRI, and labeled antibodies], targeted QDs and other bioengineered nanoparticles provide several unique features and capabilities. First, their size-dependent optical and electronic properties can be tuned continuously by changing the particle size. This size effect provides a broad range of nanoparticles for simultaneous detection of multiple cancer biomarkers. Second, nanoparticles have more surface area to accommodate a large number or different types of functional groups that can be linked with multiple diagnostic (e.g., radioisotopic or magnetic) and therapeutic (e.g.,

Positron emission tomography (PET): molecular imaging modality that uses isotopes (such as fluorine-18) for high-sensitivity detection of tumors and other diseases at medium-to-low spatial resolutions (ca. 1 mm)

Computed tomography (CT): a medical imaging modality that uses X-ray scanning and computed image reconstruction to obtain spatially resolved information on relatively dense or opaque structures such as the bone inside the body

anticancer) agents. This creates the opportunity to design multifunctional "smart" nanoparticles for multimodality imaging as well as for integrated imaging and therapy. Third, extensive research has shown that nanoparticles in the size range of 10–100 nm are accumulated preferentially at tumor sites through an effect called enhanced permeability and retention (EPR) (63–66). This effect is believed to arise from two factors: (*a*) growing tumors produce vascular endothelial growth factors (VEGFs) that promote angiogenesis and (*b*) many tumors lack an effective lymphatic drainage system, which leads to subsequent macromolecule or nanoparticle accumulation. This causes tumor-associated neovasculatures to be highly permeable, allowing the leakage of circulating macromolecules and nanoparticles into the tumor interstitium.

Mapping Sentinel Lymph Nodes and Tumor Angiogenesis

In vivo imaging with QDs has been reported for lymph node mapping, blood pool imaging, and angiogenic vessels and cell subtype isolation. Ballou and coworkers (67) injected PEG-coated QDs into the mouse blood stream and studied how the surface coating would affect their circulation time. In contrast to small organic dyes (which are eliminated from circulation within minutes after injection), PEG-coated QDs were found to stay in blood circulation for an extended period of time (half-life more than 3 h). This long-circulating feature can be explained by the unique structural properties of QD nanoparticles. PEG-coated QDs are in an intermediate size range—they are small and hydrophilic enough to slow down opsonization and reticuloendothelial uptake, but they are large enough to avoid renal filtration. Webb and coworkers took advantage of this property and reported the use of QDs and two-photon excitation to image small blood vessels (67, 68). They found that the two-photon absorption cross sections of QDs are—two to three orders of magnitude larger than that of traditional organic fluorophores. Most recently, Jain and coworkers have used of QDs and QD-doped silica beads for differentiating tumor vessels from perivascular cells and matrix (69). The results demonstrated a much clearer boundary between blood vessels and cells than that achieved by using traditional high-molecular-weight dextran.

For improved tissue penetration, Frangioni & Bawendi prepared a novel core-shell nanostructure called type II QDs (70), with fairly broad emission at 850 nm and a moderate quantum yield of ~13%. In contrast to the conventional QDs (type-I), the shell materials in type-II QDs have valence and conduction band energies lower than that of the core material. As a result, the electrons and holes are physically separated and the nanoparticles emit light at reduced energies (longer wavelengths). Their results showed rapid uptake of bare QDs into lymph nodes, and clear imaging and delineation of sentinel nodes (which are often surgically removed in patients diagnosed with breast cancer). This work points to the possibility that QD probes could be used for real-time intraoperative optical imaging, providing an in situ visual guide so that a surgeon could quickly and accurately locate and remove sentinel nodes or even small lesions (e.g., metastatic tumors), which may be difficult to identify without image guidance.

Tumor Targeting and Imaging

Akerman et al. (71) reported the use of QD–peptide conjugates to target tumor vasculatures, but the QD probes were not detected in living animals. Nonetheless, their in vitro histological results revealed that QDs homed to tumor vessels guided by the peptides and were able to escape clearance by the reticuloendothelial system (RES). Most recently, Gao et al. (49) developed a new class of multifunctional probes for simultaneous targeting and imaging of tumors in live animals. This class of QD conjugates contains an amphiphilic triblock copolymer that provides protection to aggregation and degradation in vivo and functional groups for targeting ligands for tumor antigen recognition. Addition of multiple PEG molecules provides improved biocompatibility and blood retention time. The use of an ABC triblock copolymer has solved the problems of particle aggregation and fluorescence loss previously encountered for QDs stored in physiological buffer or injected into live animals (11–18, 72). Detailed studies were reported on the in vivo behaviors of QD probes, including biodistribution, nonspecific uptake, cellular toxicity, and pharmacokinetics. Under in vivo conditions, QD probes are delivered to tumors by both a passive targeting mechanism and an active targeting mechanism. In the passive mode, macromolecules and nanometer-sized particles are accumulated preferentially at tumor sites through the EPR effect (63, 64, 66, 73). For active tumor targeting, Gao et al. (49) used antibody-conjugated QDs to target a prostate-specific cell surface antigen, PSMA. Previous research has identified PSMA as a cell surface marker for both prostate epithelial cells and neovascular endothelial cells (74). PSMA has been selected as an attractive target for both imaging and therapeutic intervention of prostate cancer. Accumulation and retention of PSMA antibody at the site of tumor growth is the basis of radioimmunoscintigraphic scanning (e.g., ProstaScint scan) and targeted therapy for human prostate cancer metastasis (75).

Correlated Optical and X-Ray Imaging

By integrating with an X-ray imaging machine, recent work by Nie and workers has also achieved optical and structural imaging on the same animal models. **Figure 3** shows correlated X-ray and fluorescence images of high-quality, deep-red QDs injected into a mouse. The superimposed X-ray and optical images showed high sensitivity in detecting small tumors with low background and high signal levels in optical imaging, while providing detailed anatomic locations of small tumors in high-resolution X-ray. This type of correlated imaging combines the unique capabilities of different imaging modalities and is becoming increasingly utilized in basic and clinical cancer research. This powerful approach should provide new insights into cancer development, progression, and metastasis in animal models.

These studies using animal models have raised new possibilities for in vivo tumor imaging and have paved the way for further development of targeted tumor imaging in cancer patients. To develop clinical applications, the current nanoparticle probes encounter several challenges, such as limited tissue penetration, lack of spatial resolution in tumor depth and location, and potential toxicity concerns. Thus, there is an

Figure 3

Combined X-ray and fluorescent imaging of near-infrared-emitting (700 nm) QD probes injected into the peritoneal cavity of a mouse. The near-infrared emission of these QDs is readily detectable above background. Images were captured sequentially with a Kodak in vivo Image Station, with 625 nm excitation for fluorescence.
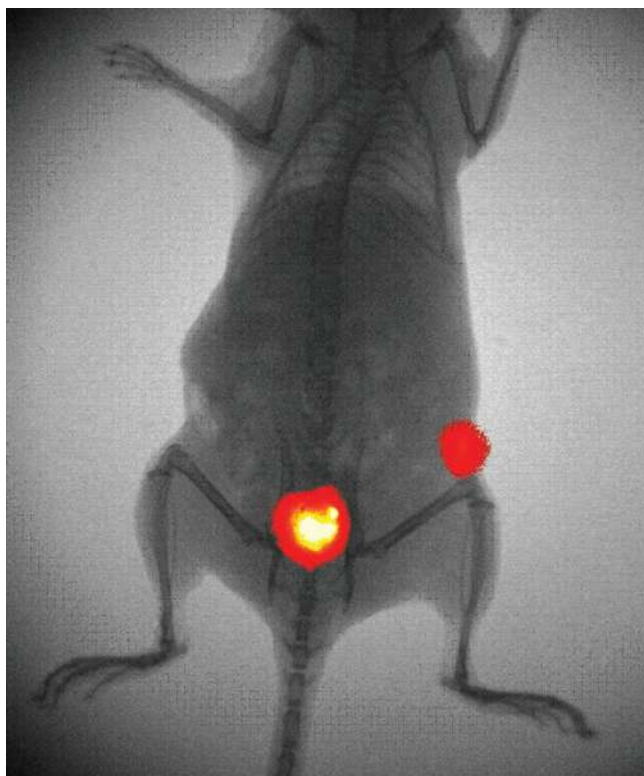
urgent need to develop broadly tunable near-infrared-emitting QDs to improve the tissue penetration depth. For clinical human applications, a major concern is likely the potential toxicity of QD probes, which has recently become a topic of considerable discussion and debate. Recent work by Derfus et al. (76) indicates that CdSe QDs are highly toxic to cultured cells under UV illumination for extended periods of time. It has also been reported that the polymer-coated QDs could be toxic if significant aggregates are formed on the cell surface (77, 78). This is not surprising because the energy of UV-irradiation is close to that of covalent chemical bond and dissolves the semiconductor particles in a process known as photolysis, which releases toxic cadmium ions into the culture medium. In the absence of UV irradiation, QDs with a stable polymer coating have been found to be essentially nontoxic to cells and animals, with no observable effects on cell division and ATP production (D. Stuart, X. Gao, and S. Nie, unpublished data). In vivo studies by Ballou and coworkers also confirmed the nontoxic nature of stably protected QDs (67). Still, there is an urgent need to study the cellular toxicity, tissue and organ clearance, and in vivo degradation mechanisms of QD probes, as well as other nanoparticle formulations used for in vivo applications. For polymer-encapsulated QDs, chemical or enzymatic degradations of the semiconductor cores are unlikely to occur. But the polymer-protected QDs might be cleared from the body by slow filtration or excretion out of the body.

This and other possible mechanisms must be carefully examined prior to any human applications in tumor or vascular imaging.

MOLECULAR CANCER DIAGNOSIS

Significant opportunities exist at the interface between biomarkers and nanotechnology for molecular cancer diagnosis. In particular, nanoparticle probes can be used to quantify a panel of biomarkers on intact cancer cells and tissue specimens, allowing a correlation of traditional histopathology and molecular signatures for the same material (see **Figure 4**). A single nanoparticle is large enough for conjugation to multiple ligands, leading to enhanced binding affinity and exquisite specificity through a multivalency effect. These features are especially important in the analysis of cancer biomarkers that are present at low concentrations or in small numbers of cells.

Multivalency effect: also known as the avidity effect, refers to a thermodynamically driven phenomenon in which the binding equilibrium constant is significantly increased when multiple ligands bind to multiple receptors simultaneously

Correlation of Biomarkers with Cancer Behavior

Most studies on QD fluorescent labeling have been carried out with cells (both live and fixed) (79–81) or freshly harvested tissues (72, 82). However, the majority of available clinical specimens are archived, formalin-fixed paraffin-embedded (FFPE) tissues that might be several decades old. Because the clinical outcomes of these tissues are already known, it is of great value to use these specimens for examining the relationship between molecular profile and clinical outcome. Compared with cells or animal tissues, archived human specimens need special treatment, such as antigen retrieval, and their background autofluorescence is generally stronger. Our group has developed highly successful procedures for QD staining of archival FFPE tissue specimens. One example is to study the epithelial-mesenchymal transition (EMT) process in the progression of prostate cancer to the bone. EMT is a normal biological mechanism first reported in embryonic development and later found to be involved in cancer metastasis (83). During EMT, cancer cells undergo phenotypical changes and become more invasive, characterized by changes in cellular adhesion molecules, particularly, an increase of N-cadherin and a loss of E-cadherin. Other important markers include the cytoskeleton proteins vimentin, cytokeratin 18, and RANKL. We have used QD-conjugated secondary antibodies for molecular profiling of two FFPE androgen-repressed prostate cancer cell lines ($ARCaP_e$ and $ARCaP_m$). These two cell lines represent two phenotypes at the two ends of the EMT process during prostate cancer progression. The $ARCaP_E$ is more epithelial-like and less invasive, whereas the $ARCaP_M$ has more mesenchymal characteristics and is more invasive (84).

QD staining studies have achieved simultaneous staining of four different biomarkers with expression profiles consistent with Western blot data (**Figure 5**). Moreover, QD staining provides spatial localization information (both inter- and intracellular), which is not possible with Western blot or other molecular biology techniques. We have also found that staining of FFPE cells requires longer incubation time (overnight at 4°C versus 1 h at room temperature) and a higher QD-secondary antibody concentration than that required for freshly fixed cells. Detailed methods
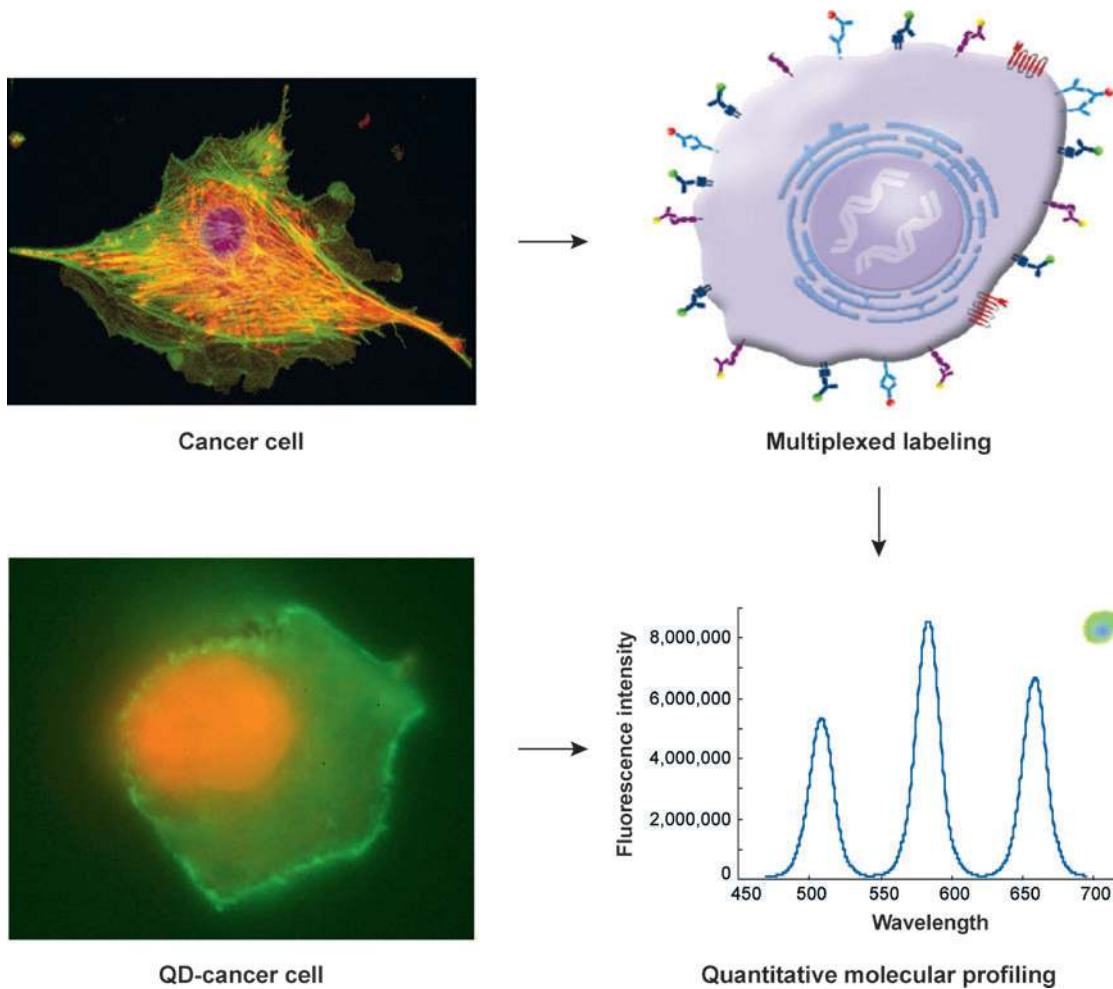
Figure 4

Schematic illustration of multiplexed detection and quantification of cancer biomarkers on intact cells or tissues with multicolor nanoparticle probes. The left-hand images show cancer cells labeled with quantum dots, and the right-hand drawings suggest how wavelength-resolved spectroscopy or spectral imaging could quantify surface and intracellular biomarkers.

and materials for QD bioconjugation, multiplexed tissue staining, and quantitative data analysis are provided in *Nature Protocols* (Nie and coworkers, 2(4):1–15, 2007).

For molecular profiling of clinical FFPE prostate specimens, we have selected four tumor antigens (mdm-2, p53, EGR-1, and p21) as a model system for technology development. These markers are known to be important in prostate cancer diagnosis and are correlated with tumor behavior (85, 86). As shown in **Figure 6**, all four markers are detected in the tissue specimens, but the autofluorescence is higher than that observed in FFPE cells. In comparison with FFPE cells, clinical tissue specimens may
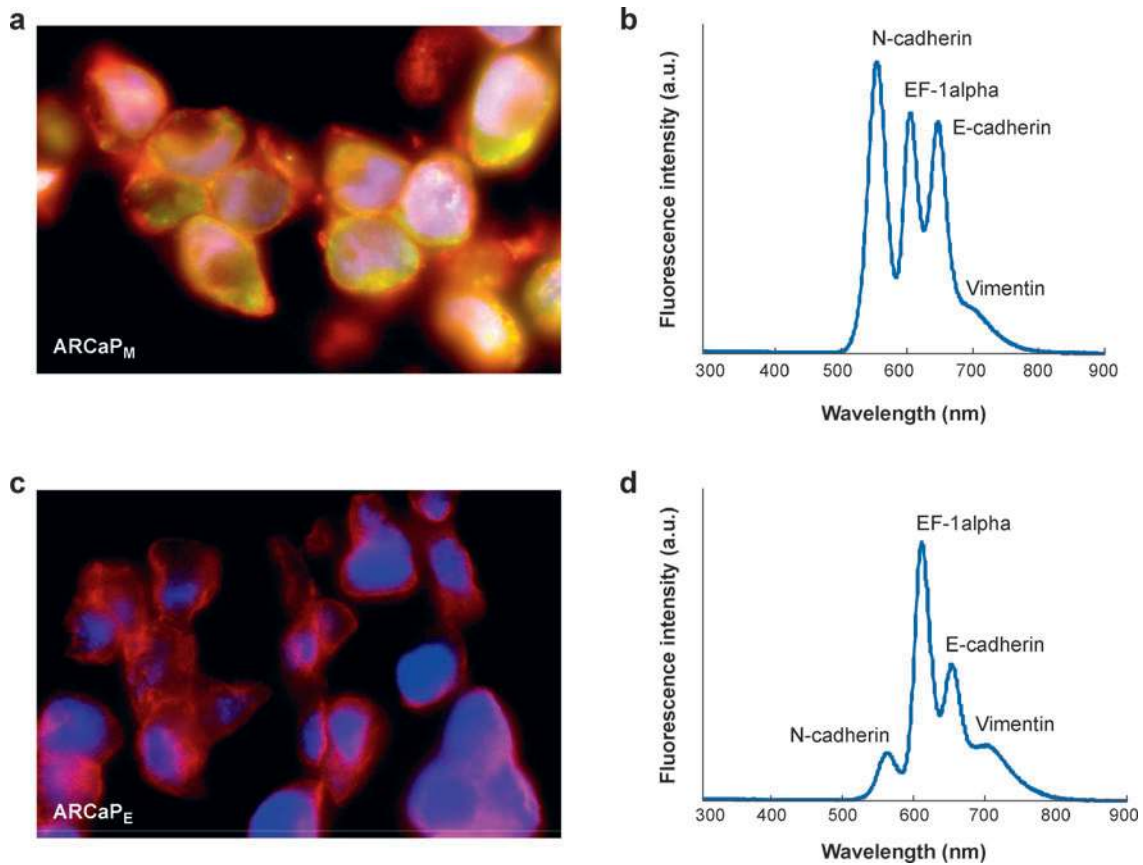
Figure 5

Multiplexed QD profiling of four tumor biomarkers using two FFPE prostate cancer cell lines (ARCaP$_e$ and ARCaP$_m$) with distinct bone-metastasis behaviors. The four markers, all associated with epithelial-mesenchymal transition (EMT), are N-cadherin, EF (elongation factor)-1alpha, E-cadherin, and vimentin, and their corresponding QD colors are 565 nm, 605 nm, 655 nm, and 705 nm, respectively. The cell nuclei were counterstained blue by DAPI, and the spectra were captured under blue excitation. (*a*) Color fluorescence image of highly metastatic prostate cancer cells (clone ARCaPm); (*b*) single-cell fluorescence spectrum obtained from image (*a*); (*c*) color fluorescence image of benign prostate cancer cells (clone ARCaPe); (*d*) single-cell spectrum obtained from image (*c*). The relative abundance of these markers is consistent with previous Western blot data. Note that individual cancer cells have heterogeneous expression patterns, and that the single-cell data in (*b*) and (*d*) are representative of a heterogeneous cell population.

require harsher antigen retrieval conditions (EDTA buffer versus citrate buffer) and generally have stronger autofluorescence. On the other hand, autofluorescence can be desirable by serving as a counterstain of tissue morphology. Autofluorescence can be separated from the QD signal by intentionally illuminating the sample to bleach it out while leaving the QDs bright enough for imaging and spectral analysis. In addition,
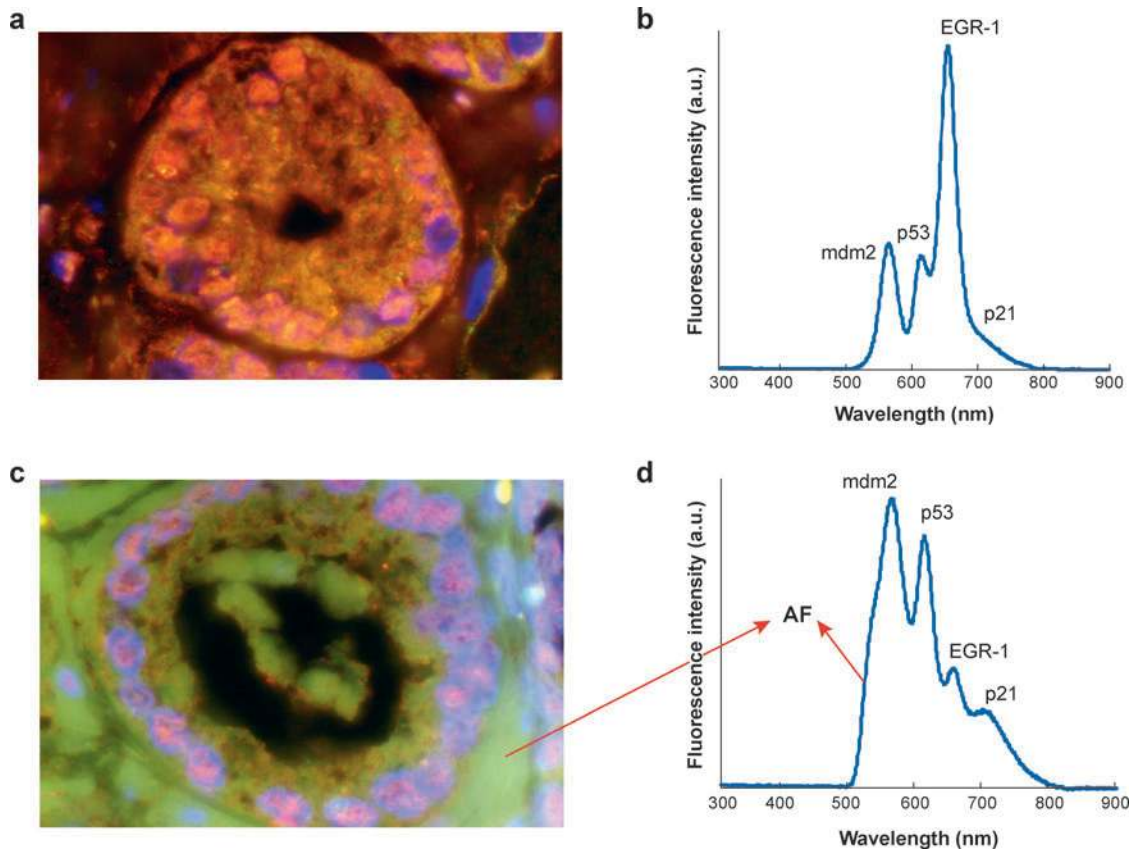
Figure 6

Multiplexed QD staining of archived FFPE clinical specimen from human prostate cancer patients and comparison between two different glands on the same tissue specimen. Four tumor biomarkers (mdm-2, p53, EGR-1, and p21) were labeled with four colors of QDs emitting at 565 nm, 605 nm, 655 nm, and 705 nm, respectively. (*a*) Color fluorescence image of QD-stained tissue specimens showing one prostate gland; (*b*) representative fluorescence spectrum obtained from individual cells in the gland (image *a*); (*c*) color fluorescence image of the same QD-stained tissue specimens showing a different gland; (*d*) representative fluorescence spectrum obtained from single cells in the second gland (image *c*). Note the distinct biomarker profiles for these two prostate glands, demonstrating the ability to resolve cellular populations in highly heterogeneous human tissue specimens. AF stands for autofluorescence and provides information on tissue morphology.

spectral unmixing algorithms can be developed in-house or obtained commercially (87) for separating background fluorescence from true QD signals. These results demonstrate the feasibility of using QDs as fluorescent labels for molecular profiling of FFPE clinical specimens. With continuous efforts in optimizing the experimental conditions, we believe that QD probes hold great promise in multiplexed molecular profiling of clinical tissue specimens and for correlation studies of biomarkers and cancer behavior.

EARLY CANCER DETECTION

Bioconjugated particles and devices are also under development for early cancer detection in body fluids such as blood and serum. These nanoscale devices operate on the principles of selectively capturing cancer cells or target proteins. The sensors are often coated with a cancer-specific antibody or other biorecognition ligands so that the capture of a cancer cell or target protein yields an electrical, mechanical, or optical signal for detection. For example, microelectrical mechanical systems (MEMS) sensors rely on the deflection of nanometer-scale cantilever beams such as carbon nanotubes and metallic oxide nanobelts, structures that are sensitive to piconewton mechnical forces. Another promising area of research is the use of nanoparticles for detection and analysis of circulating tumor cells and biomarkers in blood/serum samples (88). Vessella and coworkers (89) have demonstrated the ability to enrich for circulating cancer cells from both bone marrow aspirates and peripheral blood samples. However, the current systems are limited by the selectivity and efficiency to concentrate the rare cells for molecular assays. This is especially true for circulating cancer cells, which while present, are rarely isolated in quantities of greater than 1–2 cells per milliliter of blood. Through the combinatorial use of magnetic nanoparticles and semiconductor QDs, it is possible to increase the ability to capture and evaluate these rare circulating cancer cells. The resulting reporter signals would allow for the characterization of individual cancer cells for features associated with an aggressive phenotype (e.g., metastatic potential). The application of multiplexed nanoparticle probes would also allow for the interrogation of these cells for features related to treatment response.

Nanobarcodes

Mirkin and coworkers (90, 91) reported an innovative approach for both protein and nucleic acid detection based on biobarcode-amplification (BCA). This approach uses both colloidal gold nanoparticles and magnetic microbeads, gold nanoparticles modified with both target capture strands and bar code strands that are subsequently hybridized to bar code DNA, and magnetic microparticles modified with target capture strands. In the presence of target DNA, the gold nanoparticles and the magnetic microbeads form sandwich structures that are magnetically separated from solution and are further washed to remove the unhybridized bar code DNA. The bar codes (hundreds to thousands per target) are detected by using a colorimetric method. This integrated capture and detection technology is—four to six orders of magnitude more sensitive than standard ELISA (enzyme-linked immunosorbent assay) for proteins and offers comparable sensitvities as PCR (polymerase chain reactions) for level nucleic acid targets (91).

Nanowires

Nanowires are available in metallic, semiconductor, magnetic, oxide, and polymer compositions and are promising as ultrasmall chemical and biological sensors (92,

93). Functionalized nanowires are coated with capture ligands such as antibodies or oligonucleotides. In the presence of target molecules, the specific binding between target molecule and capture molecule generates an immediate conductivity change within the nanowire that can be measured. Hahm et al. (94) used silicon nanowire for ultrasensitive and selective detection of DNA. The surface of this nanowire device was coated with peptide nucleic acid (PNA) ligands for recognizing a mutation site in the cystic fibrosis transmembrane receptor gene. The achieved detection limit is on the order of 10 femtomolar (10×10^{-15} M). The same group has also developed nanowire arrays for multiplexed cancer biomarker detection (95), which consist of many individual nanowires each coated with a distinct surface receptor. These nanowire arrays allow simultaneous incorporation of control nanowires, which enables discrimination against false positives; they are also capable of selective and sensitive multiplexed detection of cancer biomarkers such as PSA, PSA-α1-antichymotrypsin, carcinoembryonic antigen, and mucin-1 in undiluted serum samples (95).

Carbon Nanotubes

Another type of nanodevice for biomarker detection is the carbon nanotube (CNT) (96). Using single-walled carbon nanotubes as high-resolution atomic force microscopy (AFM) tips, Woolley et al. (97) showed that specific sequences of kilobase-size DNA can be selectively detected from single-base mismatch sequences. Specifically, target DNA fragments were first hybridized with labeled (for instance, strepavidin-labeled) oligonucleotides, and then AFM was used to directly detect the presence and special location of the labels. This technique enabled the simple and direct detection of specific haplotypes that code for genetic disorders such as cancer. CNT-modified electrodes can amplify the electrochemical signal of guanuine bases, which has been used by Wang et al. (98) for label-free electrochemical detection of DNA at nanomolar concentrations. More recent work has utilized CNTs coated with alkaline phosphatase as labels for amplified DNA and protein detection (99). In this assay, CNTs play a dual amplification role in the recognition and transduction events, namely as carriers for numerous enzyme tags and for accumulating products of the enzymatic reaction.

TARGETED CANCER THERAPY

As noted above, most current anticancer agents do not greatly differentiate between cancerous and normal cells, leading to systemic toxicity and adverse effects. Consequently, systemic applications of these drugs often cause severe side effects in other tissues (such as bone marrow suppression, cardiomyopathy, and neurotoxicity), which greatly limits the maximal allowable dose of the drug. In addition, rapid elimination and widespread distribution into nontargeted organs and tissues requires the administration of a drug in large quantities, which is not economical and often complicated owing to nonspecific toxicity. Nanotechnology offers a more targeted approach and could thus provide significant benefits to cancer patients. In fact, the use of nanoparticles for drug delivery and targeting is likely one of the most exciting and clinically

important applications of cancer nanotechnology. In this section, we discuss different targeting strategies for nanoscale drug delivery systems.

Passive Targeting

Rapid vascularization in fast-growing cancerous tissues is known to result in leaky, defective architecture and impaired lymphatic drainage. This structure allows an EPR effect (63, 64, 66, 73), resulting in the accumulation of nanoparticles at the tumor site (**Figure 7**). For such a passive targeting mechanism to work, the size and surface properties of drug delivery nanoparticles must be controlled to avoid up-take by the reticuloendothelial system (RES) (100). To maximize circulation times and targeting ability, the optimal size should be less than 100 nm in diameter and the surface should be hydrophilic to circumvent clearance by macrophages. A hy-drophilic surface of the nanoparticles safeguards against plasma protein adsorption and can be achieved through hydrophilic polymer coatings such as PEG, poloxamines, poloxamers, polysaccharides, or through the use of branched or block amphiphilic copolymers (101–104). The covalent linkage of amphiphilic copolymers (polylactic
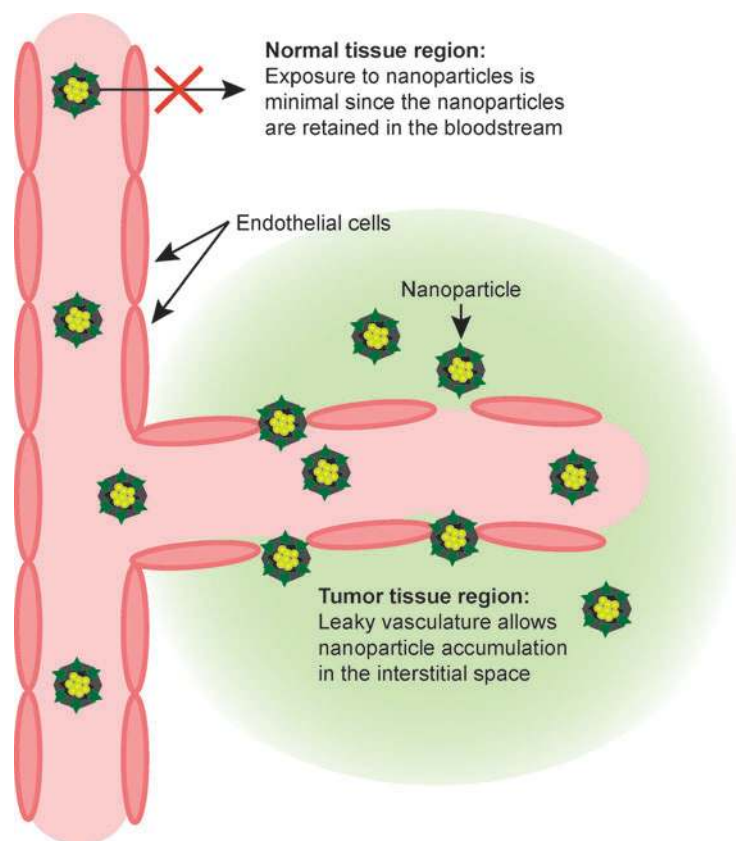

Figure 7

Schematic diagrams showing enhanced permeability and retention of nanoparticles in tumors. Normal tissue vasculatures are lined by tight endothelial cells, thereby preventing nanoparticle drugs from escaping or extravasation, whereas tumor tissue vasculatures are leaking and hyperpermeable allowing preferential accumulation of nanoparticles in the tumor interstitial space (called passive nanoparticle tumor targeting).

acid, polycaprolactone, polycyanonacrylate chemically coupled to PEG) is generally preferred, as it avoids aggregation and ligand desorption when in contact with blood components.

An alternative passive targeting strategy is to utilize the unique tumor environment in a scheme called tumor-activated prodrug therapy. The drug is conjugated to a tumor-specific molecule and remains inactive until it reaches the target (105). Overexpression of the matrix metalloproteinase (MMP) MMP-2 in melanoma has been shown in a number of preclinical as well as clinical investigations. Mansour et al. (106) reported a water-soluble maleimide derivative of doxorubicin (DOX) incorporating an MMP-2-specific peptide sequence (Gly-Pro-Leu-Gly-Ile-Ala-Gly-Gln) that rapidly and selectively binds to the cysteine-34 position of circulating albumin. The albumin-DOX conjugate is efficiently and specifically cleaved by MMP-2, releasing a DOX tetrapeptide (Ile-Ala-Gly-Gln-DOX) and subsequently DOX. pH and redox potential have been also explored as drug release triggers at the tumor site (107). Another passive targeting method is the direct local delivery of anticancer agents to tumors. This approach has the obvious advantage of excluding the drug from the systemic circulation. However, administration can be highly invasive, as it involves injections or surgical procedures. For some tumors, such as lung cancers, that are difficult to access, the technique is nearly impossible to use.

Active Targeting

Active targeting is usually achieved by conjugating to the nanoparticle a targeting component that provides preferential accumulation of nanoparticles in the tumor-bearing organ, in the tumor itself, individual cancer cells, or intracellular organelles inside cancer cells. This approach is based on specific interactions, such as lectin-carbohydrate, ligand-receptor, and antibody-antigen (108). Lectin-carbohydrate is one of the classic examples of targeted drug delivery (109). Lectins are proteins of nonimmunological origin, capable of recognizing and binding to glycoproteins expressed on cell surfaces. Lectin interactions with certain carbohydrates are very specific. Carbohydrate moieties can be used to target drug delivery systems to lectins (direct lectin targeting), and lectins can be used as targeting moieties to target cell surface carbohydrates (reverse lectin targeting). However, drug delivery systems based on lectin-carbohydrate have mainly been developed to target whole organs (110), which can pose harm to normal cells. Therefore, in most cases the targeting moiety is directed toward specific receptors or antigens expressed on the plasma membrane or elsewhere at the tumor site.

The overexpression of receptors or antigens in many human cancers lends itself to efficient drug uptake via receptor-mediated endocytosis (**Figure 8**). Because glycoproteins cannot remove polymer-drug conjugates that have entered the cells via endocytosis (111, 112), this active targeting mechanism provides an alternative route for overcoming multiple drug resistance (MDR) (113–118).

The cell surface receptor for folate is inaccessible from the circulation to healthy cells owing to its location on the apical membrane of polarized epithelia, but it is overexpressed on the surface of various cancers, including ovary, brain, kidney, breast, and
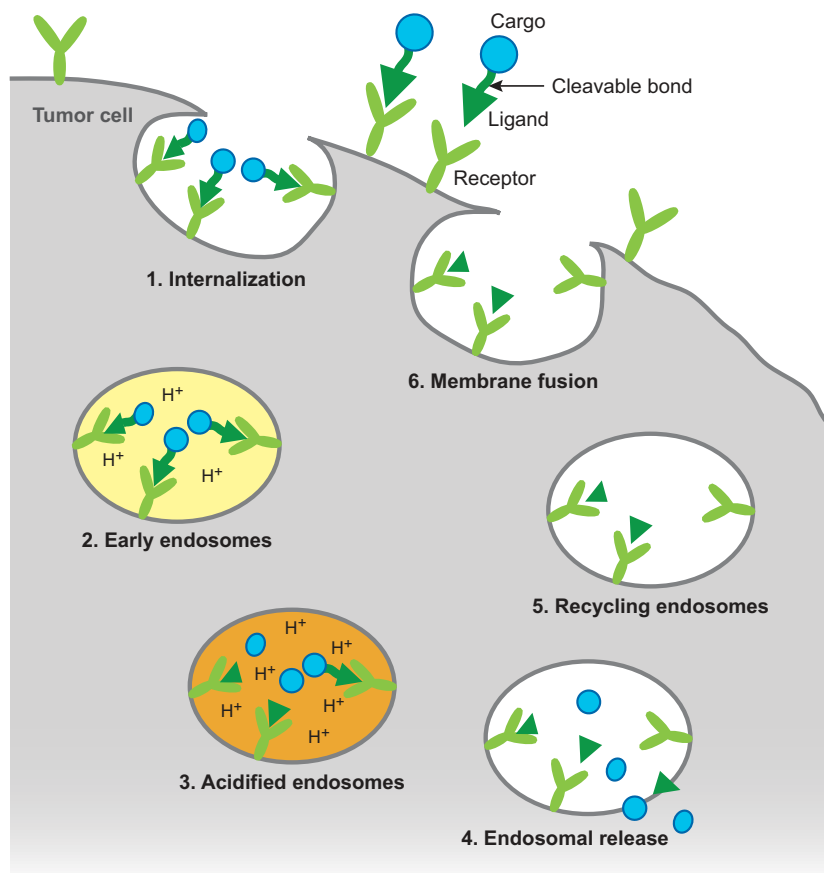
Figure 8

Nanoparticle drug delivery and targeting using receptor-mediated endocytosis. The nanoparticle drug is internalized by tumor cells through ligand-receptor interaction. Depending on the design of the cleavable bond, the drug will be released intracellularly on exposure to lysosomal enzymes or lower pH.

lung malignancies (119, 120). Surface plasmon resonance studies revealed that folate-conjugated PEGylated cyanoacrylate nanoparticles had a tenfold higher affinity for the folate receptor than free folate did (121). Folate receptors are often organized in clusters and bind preferably to the multivalent forms of the ligand. Furthermore, confocal microscopy demonstrated selective uptake and endocytosis of folate-conjugated nanoparticles by tumor cells bearing folate receptors. Interest in exploiting folate receptor targeting in cancer therapy and diagnosis has rapidly increased, as attested by many conjugated systems, including proteins, liposomes, imaging agents, and neutron activation compounds (119, 120).

Nanoparticle Drugs

Nanotechnology is beginning to change the scale and methods of drug delivery (**Figure 9**). Therapeutic and diagnostic agents can be encapsulated, covalently attached, or adsorbed onto nanoparticles. These approaches can easily overcome drug
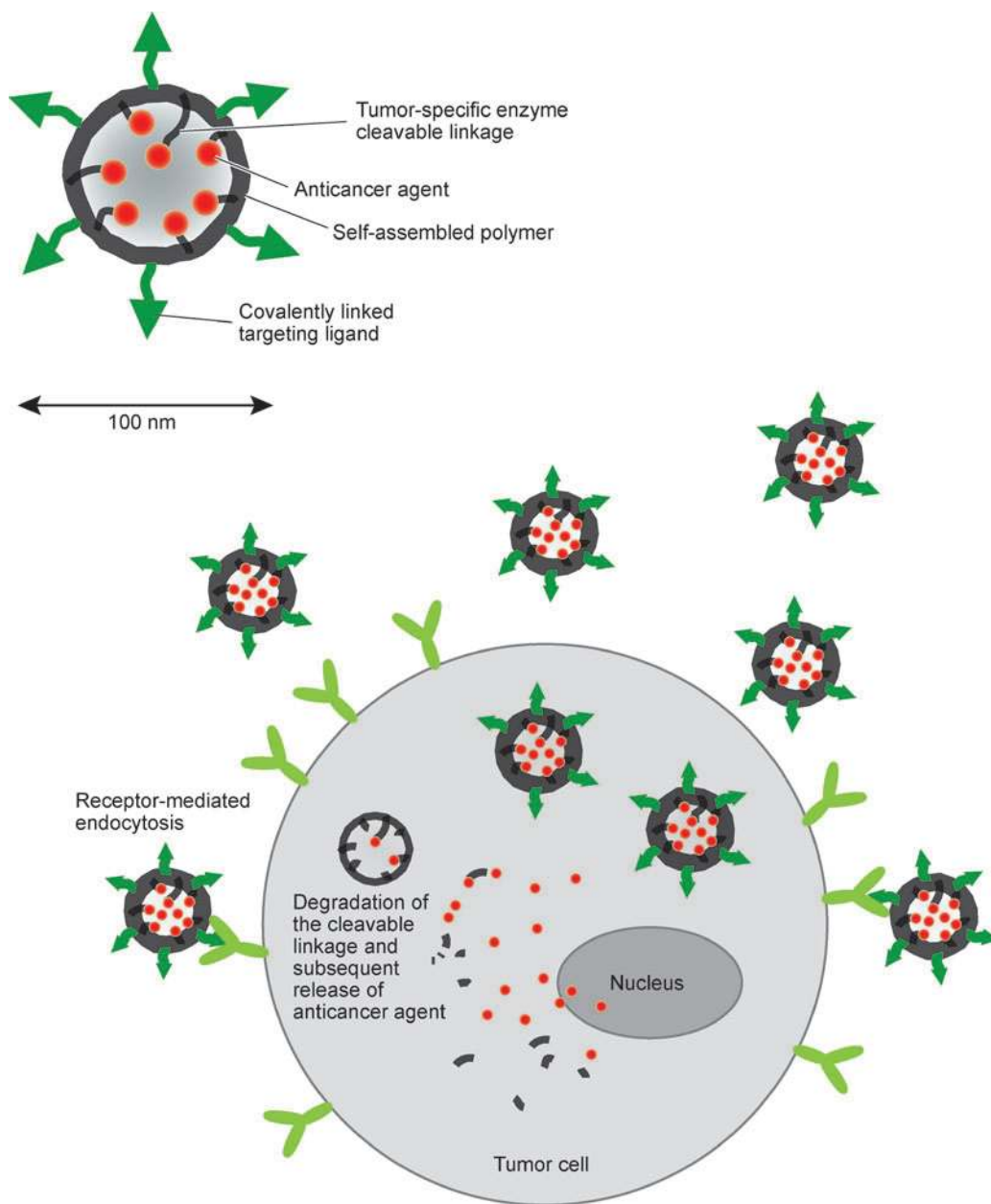
Figure 9

Illustration showing self-assembled polymeric nanoparticles with dual tumor-targeting and therapeutic functions (*upper panel*) and delivery of the nanoparticle drugs by receptor-mediated endocytosis and controlled drug release inside the cytoplasm (*lower panel*).

solubility issues, which has significant implications because more than 40% of active substances being identified through combinatorial screening programs are poorly soluble in water (122). Conventional and most current formulations of such drugs are frequently plagued with problems such as poor and inconsistent bioavailability. The widely used attempt at enhancing solubility is to generate a salt. For nonionizable compounds, micronization, soft-gel technology, cosolvents, surfactants, or complexing agents have been used (123). Because it is faster and more cost effective to reformulate the drug than to develop a new one, a broadly based technology applicable to poorly water-soluble drugs could make a tremendous impact.

For decades, researchers have been developing new anticancer agents and new formulations for delivering chemotherapy drugs (112). Paclitaxel (Taxol^{TM}) is one of the most widely used anticancer drugs in the clinic. It is a microtubule-stabilizing agent that promotes tubulin polymerization, disrupting cell division and leading to cell death (124, 125). It displays neoplastic activity against primary epithelial ovarian carcinoma and breast, colon, and lung cancers. Because it is poorly soluble in aqueous solution, the formulation available currently is Chremophor EL (polyethoxylated castor oil) and ethanol (126). In a new formulation approach used in Abraxane^{TM}, recently approved by the FDA to treat metastatic breast cancer, paclitaxel was conjugated to albumin nanoparticles (127, 128). The formulation is very effective in circumventing side effects of the highly toxic Chremophor EL, which include hypersensitivity reactions, nephrotoxicity, and neurotoxicity (126, 129). Although the SPACR (secreted protein, acidic, cysteine-rich, also called osteonectin) protein is

FDA-APPROVED NANOPARTICLE DRUG—ABRAXANE

The Food and Drug Administration (FDA) recently approved Abraxane^{TM}, an albumin-paclitaxel (Taxol^{TM}) nanoparticle for the treatment of metastatic breast cancer. A Phase I clinical trial determined that the maximum tolerated dose (MTD) of single-agent albumin-bound paclitaxel every 3 weeks was 300 mg/m^2 in patients with solid tumors (breast cancer and melanoma). A second Phase I trial, reported at the 2004 ASCO Annual meeting, demonstrated 5 responses among 39 pretreated patients with advanced solid tumors, including 1 response in a patient with NSCLC, 3 responses in patients with ovarian cancer, and 1 in breast cancer. The dose-limiting toxicity was myelosuppression, the MTD was 270 mg/m^2, and premedication was not required. Subsequent use of Abraxane in both Phase II and Phase III trails proved that this new formulation was far superior to Taxol^{TM}. In a randomized, open-labeled trial of 454 patients with metastatic breast cancer, the overall response rate for ABI-007 was 33%, compared with 19% for Taxol^{TM}. Median time to progression was 21.9 weeks for ABI-007, versus 16.1 weeks for Taxol^{TM}. Overall side effects were fewer ABI-007, even though it delivered a 50% higher dose of the active agent Taxol^{TM}.
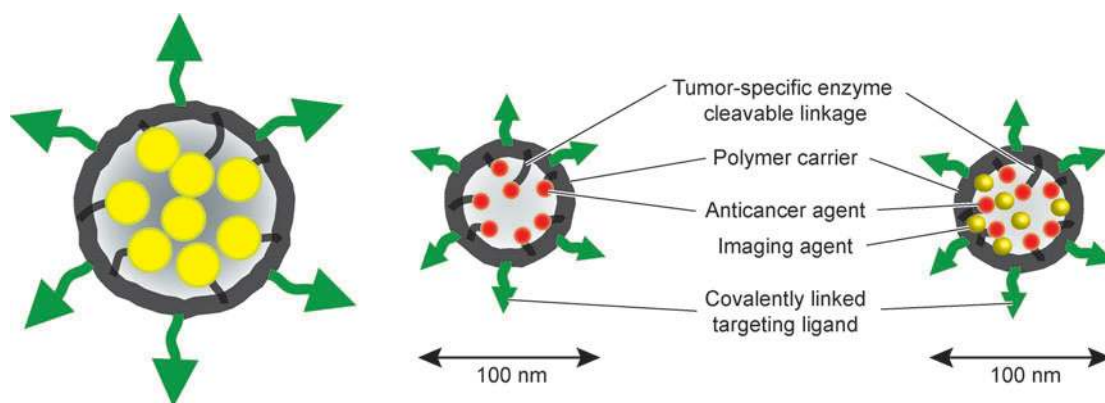
Figure 10

Multifunctional nanoparticles for integrated cancer imaging and therapy. A truly exciting feature of cancer nanotechnology is that drug delivery, treatment efficacy, and toxicity could be monitored by using embedded imaging agents.

believed to improve albumin drug uptake, this nanoparticulate drug still exhibits significant side effects (see FDA-Approved Nanoparticle Drug—Abraxane).

For enhanced tumor-specific targeting, the differences between cancerous cells and normal cells may be exploited. By virtue of their small size, nanoparticles entail a high surface area that not only paves the way for more efficient drug release but also a better strategy for functionalization. There is a growing body of knowledge of unique cancer markers thanks to recent advances in proteomics and genomics. They form the basis of complex interactions between bioconjugated nanoparticles and cancer cells. Carrier design and targeting strategies may vary according to the type, developmental stage, and location of cancer (130). There is much synergy between imaging and nanotechnology in biomedical applications. Many of the principles used to target delivery of drugs to cancer may also be applied to target imaging and diagnostic agents to enhance detection sensitivity in medical imaging. With engineered multifunctional nanoparticles (**Figures 10**), the full in vivo potential of cancer nanotechnology in targeted drug delivery and imaging can be realized.

FUTURE DIRECTIONS

Nanotechnology has become an enabling technology for personalized oncology in which cancer detection, diagnosis, and therapy are tailored to each individual's tumor molecular profile, and also for predictive oncology in which genetic/molecular markers are used to predict disease development, progression, and clinical outcomes. In recognition of its potential impact in cancer research, the U.S. National Cancer Institute (NCI) has recently funded eight national Centers of Cancer Nanotechnology Excellence (CCNE). Looking into the future, there are a number of research themes or directions that are particularly promising but require concerted effort

for success. The first direction is the design and development of nanoparticles with monofunctions, dual functions, three functions, or multiple functions. For cancer and other medical applications, important functions include imaging (single or dual-modality), therapy (single drug or combination of two or more drugs), and targeting (one or more ligands). With each added function, nanoparticles could be designed to have novel properties and applications. For example, binary nanoparticles with two functions could be developed for molecular imaging, targeted therapy, or for simultaneous imaging and therapy (but without targeting). Bioconjugated QDs with both targeting and imaging functions will be used for targeted tumor imaging and molecular profiling applications. Conversely, ternary nanoparticles with three functions could be designed for simultaneous imaging and therapy with targeting, targeted dual-modality imaging, or targeted dual-drug therapy. Quaternary nanoparticles with four functions can be conceptualized in the future to have the abilities of tumor targeting, dual-drug therapy, and imaging. The second direction is nanoparticle molecular profiling (nanotyping) for clinical oncology; that is, the use of bioconjugated nanoparticle probes to predict cancer behavior, clinical outcome, and treatment response and to individualize therapy. This should start with retrospective studies of archived specimens because the patient outcome is already known for these specimens. The key hypotheses to be tested are that nanotyping a panel of tumor markers will allow more accurate correlations than single tumor markers, and that the combination of nanotyping tumor gene expression and host stroma are both important in defining the aggressive phenotypes of cancer as well as determining the response of early stage disease to treatment (chemotherapy, radiation, or surgery). The third important direction is to study nanoparticle distribution, excretion, metabolism, and pharmacodynamics in in vivo animal models. These investigations will be very important in the development of nanoparticles for clinical applications in cancer imaging or therapy.

SUMMARY POINTS

1. Nanometer-sized particles have novel optical, electronic, magnetic, or structural properties and are currently under intense development for applications in cancer, cardiovascular diseases, and degenerative neurological disorders such as Alzheimer's disease.

2. Quantum dots are just one type of nanoparticle with novel optical properties such as size-tunable emission, improved signal brightness, resistance against photobleaching, and simultaneous excitation of multiple fluorescence colors.

3. Dual-modality and multifunctional probes are being developed by attaching molecular moieties with imaging, therapeutic, and targeting functions to nanostructured scaffolds. These integrated nanoparticle probes may allow simultaneous imaging and therapy of tumors and cardiovascular plaques in live animal models.

4. Antibody-conjugated multicolor quantum dots have been used for multi-plexed molecular profiling of cancer cells and clinical tissue specimens, and for correlation of a panel of 4–5 biomarkers with cancer behavior and patient outcome.

5. Bionanobarcodes, nanocantilevers, and nanowires are promising technologies for early cancer detection and screening in blood and serum samples.

6. Targeted nanoparticle drugs offer significant advantages in improving cancer therapeutic efficacy and simultaneously reducing drug toxicity.

7. Dual- and multimodality nanoparticles are being developed by attaching molecular moieties with imaging, therapeutic, and targeting functions to nanometer-scaled scaffolds for simultaneous imaging and therapy of tumors.

8. Future work needs to address the potential long-term toxicity, degradation, and metabolism of nanoparticle agents, to identify and develop new biomarker-probe systems, and to develop multifunctional nanoscale platforms for integrated imaging, detection, and therapy.

DISCLOSURE STATEMENT

The authors are not aware of any biases that might be perceived as affectin the objectivity of this review.

ACKNOWLEDGMENTS

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