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MINI REVIEW

Nanomedicine for drug delivery and imaging: A promising avenue for cancer therapy and diagnosis using targeted functional nanoparticles

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The diagnosis and treatment of cancer or tumor at the cellular level will be greatly improved with the development of techniques that enable the delivery of analyte probes and therapeutic agents into cells and cellular compartments. Organic and inorganic nanoparticles that interface with biological systems have recently attracted widespread interest in the fields of biology and medicine. The new term nanomedicine has been used recently. Nanoparticles are considered to have the potential as novel intravascular or cellular probes for both diagnostic (imaging) and therapeutic purposes (drug/gene delivery), which is expected to generate innovations and play a critical role in medicine. Target-specific drug/gene delivery and early diagnosis in cancer treatment is one of the priority research areas in which nanomedicine will play a vital role. Some recent breakthroughs in this field recently also proved this trend. Nanoparticles for drug delivery and imaging have gradually been developed as new modalities for cancer therapy and diagnosis. In this article, we review the significance and recent advances of gene/drug delivery to cancer cells, and the molecular imaging and diagnosis of cancer by targeted functional nanoparticles.

Key words: targeting nanoparticles; surface modification; drug delivery; gene transfer; molecular imaging; cancer therapy

Nanotechnology, although not a new concept, has gained significant momentum in recent years. The prefix ''nano'' means to one-billionth. In the metric scale of linear measurements, a nanometer is one-billionth of a meter. Primarily in the materials science standard, the term ''nanotechnology'' is now commonly used to refer to the fabrication of new materials with nanoscale dimensions between 1 and 100 nm.[1] However, with its development, the scope of this definition also expanded. Nanoparticles of different sizes have different biomedical purposes. In physics and electrical engineering, nanotechnology is associated with quantum behavior, and the behavior of electrons and photons in nanoscale structures. Recently, Whitesides reviewed and interpreted the relationship among nanotechnology, chemistry, and biology.[2]

Nanobiotechnology is defined by science's growing ability to work at the molecular level, atom by atom, combining biological materials and the rules of physics, chemistry, and genetics to fabricate minute synthetic structures.[3,4] The ultimate goal of nanobiotechnology is to develop a highly functional system of biosensors, electronic circuits, nanosized microchips, molecular ''switches,'' and even tissue analogs for growing skin, bones, muscle, and other organs of the body. The application of nanotechnology to disease treatment, diagnosis, monitoring, and to the control of biological systems has recently been referred to as ''nanomedicine'' by National Institutes of Health in USA. Research into the rational delivery and targeting of pharmaceutical, therapeutic, and diagnostic agents is at the forefront of projects in nanomedicine.[5,6] Nanosystems, which can be designed to have different compositions, have unique physical and biological properties that might be used to overcome the limitations of molecular imaging and gene/drug delivery in recent years.[7–10] Some of these systems, such as quantum dots (Qdots), silica nanoparticles, dendrimers, micelles, molecular conjugates, liposomes, and ultrasound microbubbles, have been extensively investigated for imaging and drug/gene delivery applications.[8,11–14] Moreover, after the development of multifunctionalized nanoparticles as shown in Figure 1, their applications in medicine will be unprecedented.[3] Cancer is among the top three ''killers'' in modern society, which include cardiovascular diseases, and cancer patients are not very satisfied with the current treatment options. Regarding the present therapy methods, including surgery, chemotherapy, and radiation therapy, many forms of cancer are treatable by these therapies; however, today's therapies sometimes have significant toxicities, and side effects are common. For instance, in the process of killing cancer cells, chemotherapeutic agents also damage healthy tissues. Thus, the important first step in improving treatment regimens is better harnessing utilization of the potency of therapeutic agents (drugs or genes) by more effectively targeting them to tumor tissues. The goal of nanomedicine is to develop safer and more effective therapeutic and diagnostic modalities. Because of the size and supramolecular structure of nanoparticle, technologies utilizing nanoparticles have much potential for improving cancer therapy. A primary attribute of nanoparticles delivery systems is their potential to enhance the tumor accumulation of anticancer agents in tumor cells than in healthy tissues.[15,16] Although an effective cancer therapy is still problematic in recent years, nanobiotechnology developed very rapidly with some exciting achievements, namely, nanoparticles as drug carriers or imaging tools, which ushers in a dawn and a new avenue for cancer therapy and diagnosis.[17,18] In this article, we review recent advances in gene/drug delivery to cancer therapy and their significance, and molecular imaging and diagnostic techniques using targeted functional nanoparticles.

Nanoparticles/nanocapsules as drug delivery carriers to cancer

A conventional drug delivery system using microcapsules or nanoparticles is shown in Figure 2.[19] A drug can be encapsulated in particles or attached to the surfaces of capsules. Side effects are due to toxicities to sensitive normal cells because the therapies are not selective for target cells. Recently, targeted therapeutics in nanome-

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dicine have been widely explored. Drug targeting by nanoparticles or nanocapsules offers the following enormous advantages,[9,20,21] as examples: reduces dosage, ensures the pharmaceutical effects, and minimizes side-effects; protects drugs against degradation and enhances drug stability.[22] Nanoparticles can penetrate through small capillaries and are taken up by cells, which allows efficient drug accumulation at target sites. A sustained and controlled release of drugs at target sites over a period of days or even weeks is possible. To date, many types of drug delivery nanosystems have been developed, for example, polymeric nanoparticles of poly(D,L-lactide-*co*-glycolide) (PLGA), liposomes, dendrimers, micelles, and silica nanoparticles.[23–25] We have been investigating silica nanocapsules for a controlled release of drugs. We successfully prepared hollow mesoporous silica nanocapsules (HMSNs) with very thin shells in the range of 3–10 nm (Fig. 3), thereby obtaining a more than 70% ratio of the hollow core to HMSNs, which ensures encapsulation of a great amount of drugs. HMSNs were used as drug carriers to investigate fluorescein isothiocyanate (FITC, as a model drug) release *in vitro*. FITC in silica nanocapsules is released more slowly than free FITC. The FITC release peaks ~1.5 hr (Fig. 4). Research on the application of HMSNs as a targeted drug delivery system for cancer treatment will be carried out in the future.

Although the delivery of anticancer therapeutic agents to solid tumors is still problematic, nanoparticles as drug carriers are an attractive alternative drug delivery system because they apparently target tumors and have limited toxicity to normal tissues.[26] Many of these drug delivery approaches take advantage of the unique pathophysiology of the tumor vasculature. In contrast to normal tissue, tumors contain a high density of abnormal blood vessels that are dilated and poorly differentiated, with a chaotic architecture and aberrant branching.[27,28] Subsequently, various functions of the tumor vasculature were found to be impaired; these impaired functions account for the higher concentration of plasma proteins detected in tumor tissues than in normal tissues.[29] This is due to an enhanced permeability and retention effect, which result from the combination of an increased permeability of tumor blood vessels and a decreased rate of clearance caused by the lack of functional lymphatic vessels in the tumor, and results in an increased accumulation of macromolecules in tumors (Fig. 5).[29–31] These findings support the use of nanoparticles in tumor diagnosis and therapy as carriers, because they passively accumulate in solid tumors after their intravenous administration.

As is known, a major difficulty is to destroy tumor cells without destroying normal tissues during cancer treatment. As mentioned in the above section, radiotherapy and chemotherapy cause some severe side effects. Recently, the general issue ''Drugs on Target'' was brought into focus by Langer in 2001.[10] Several approaches to improving the selective toxicity of anticancer therapeutics are being pursued presently.[32–34] The most commonly used method is

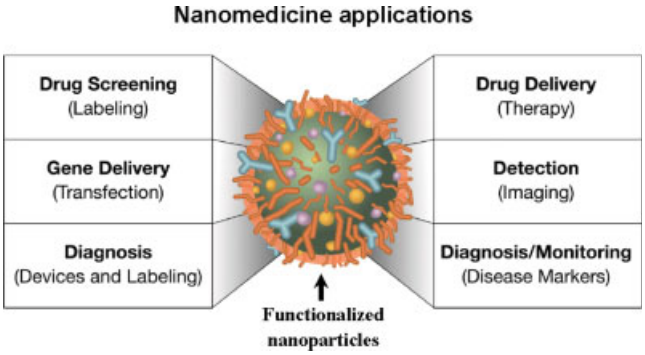

FIGURE 1 – Applications and research targets of nanomedicine. Nanoparticles have been designed with chemically modifiable surfaces onto which various ligands attach, which can turn these nanomaterials into biosensors, molecular-scale fluorescent tags, imaging agents, targeted molecular delivery vehicles, and other useful biological tools (Reproduced with some modifications from Ref. 3, with permission from ©HighWire, and McNeil). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

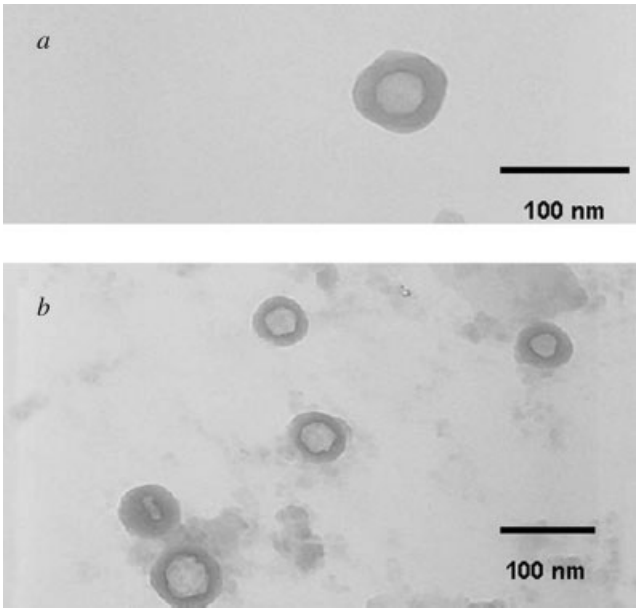

FIGURE 3 – Transmission electron micrographs of silica nanocapsules: (*a*) diameter ~50 nm, (*b*) diameter 60 nm. Core-shell nanoparticles of Au@silica were synthesized using gold nanoparticles as templates. The porosity of silica shells is regulated by adding 3-aminopropyltrimethoxysilane. Hollow mesoporous silica nanocapsules (HMSNs) are prepared using sodium cyanide to dissolve gold cores, then rinsed in Milli-Q water to remove toxic sodium cyanide.

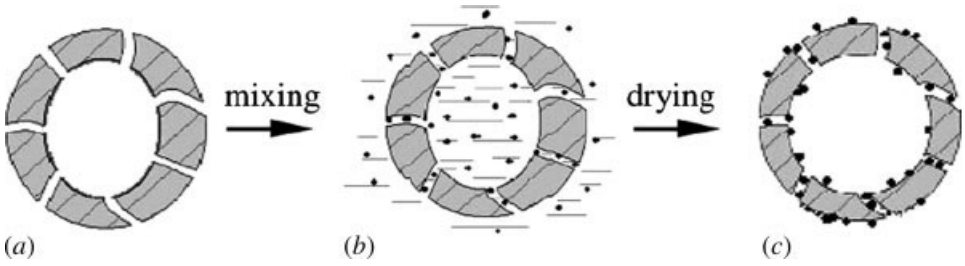

FIGURE 2 – Representative preparation of porous hollow silica nanocapsules (PHSNP) for cefradine delivery (an antibacterial chemical) by Chen *et al.*[19] (*a*) PHSNP. Solid nanoparticles are synthesized using $CaCO_3$ as the template, then the $CaCO_3$ template is dissolved in HCl; (*b*) Mixture of cefradine and PHSNP in suspension. The suspension was stirred for 1 day, and then rinsed with acetone to remove nontrapped cefradine; (*c*) PHSNP entrapped with cefradine after drying. The powder with the trapped drug was dried in vacuum (Reproduced from Ref. 19, with permission from ©Elsevier, and Chen *et al.*).

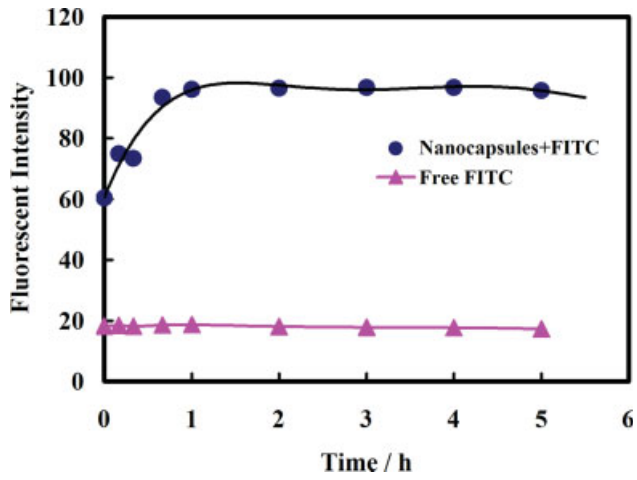

FIGURE 4 – Time course of FITC release from silica nanocapsules (50 nm) by detecting fluorescence intensity. Free FITC solution and silica nanocapsule solution with FITC are all stirred for 4 h and washed using ultrafiltration membrane with Milli-Q water three times, and then the fluorescence intensity is measured. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

antibody- or ligand-mediated targeting of anticancer therapeutics. The basic principle that underlies ligand-targeted therapeutics is that the selective delivery of antineoplastic drugs to cancer cells or cancer-associated tissues such as tumor vasculature can be enhanced by associating the drugs with molecules that bind to antigens or receptors that are either uniquely expressed or overexpressed on target cells compared with normal tissues. This allows the specific delivery of drugs to cancer cells.[35–39]

PLGA is a very good material for generating PLGA nanoparticles, which are biocompatible and degradable with no toxicity. Cegnar *et al*. have investigated the use of PLGA nanoparticles containing cystatin, a potential anticancer drug inhibiting the tumor-associated activity of intracellular cysteine proteases cathepsins,[23] as a carrier system to regress tumor growth, and showed that PLGA nanoparticles are useful for a rapid delivery of protein inhibitors into tumor cells, enabling an effective inhibition of the intracellular proteolysis. McCarthy *et al*. have recently synthesized another new type of a carrier system, that is, PLGA nanoparticles encapsulating the photosensitizer *meso*-tetraphenylporpholactol.[40] After cellular internalization, the photosensitizer is released from the nanoparticles and becomes highly phototoxic. They irradiated these nanoparticles with visible light, resulting in a cell-specific killing of several cancer cell lines, such as 9L glioblastoma cells and B16 melanoma cells. Farokhzad *et al*.[41] developed docetaxel-encapsulated pegylated PLGA nanoparticle-aptamer bioconjugates. These bioconjugated nanoparticles bind to the prostate-specific

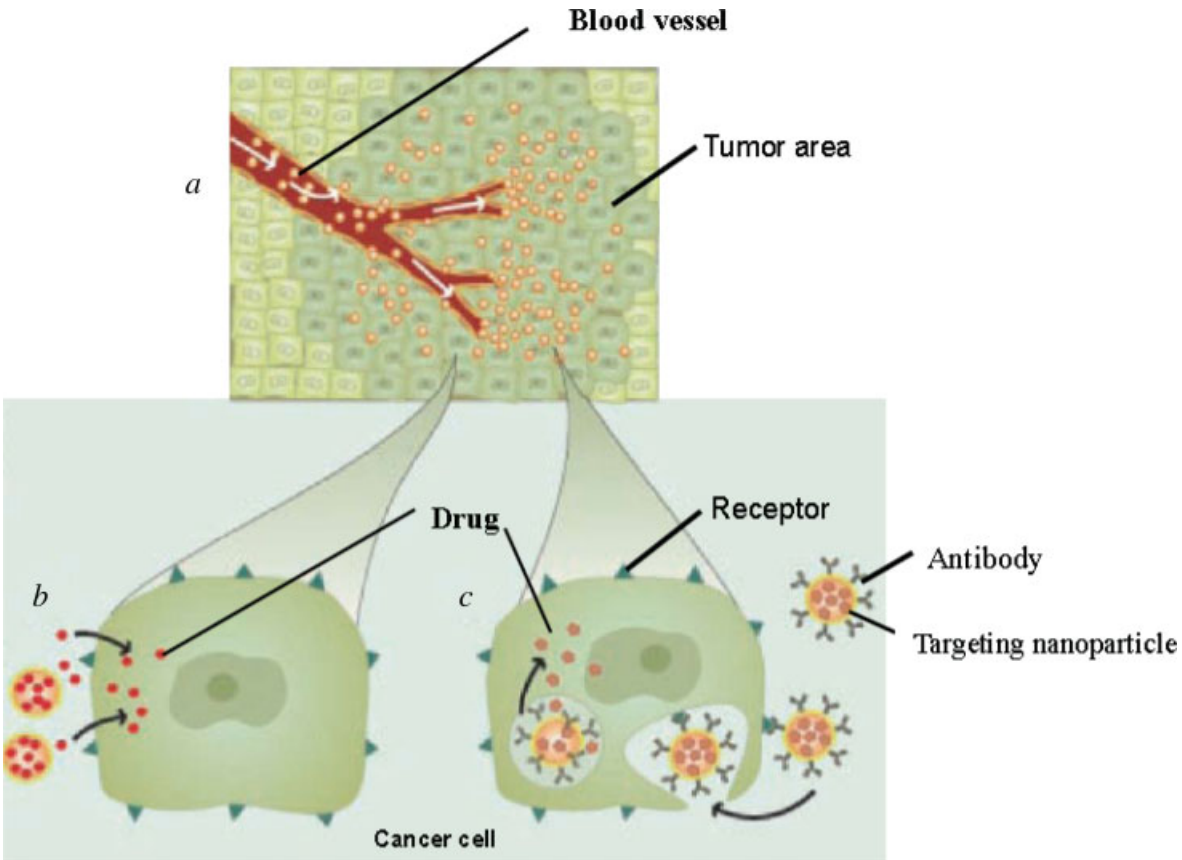

FIGURE 5 – Schematic diagram showing the passive or ligand-targeted accumulation of drug delivery system in tumor therapy through the enhanced permeability and retention effect. (*a*) Nanoparticles containing an anticancer drug extravasate from the blood through gaps in vascular endothelial cells and accumulate in tumor tissue, but not in normal tissue. (*b*) Drug is released from the nanoparticles in the vicinity of tumor cells and taken up into the cells. (*c*) Ligand-targeted nanoparticles containing anticancer drugs, or nucleic acid-based therapeutics such as plasmid DNA or antisense oligonucleotides, bind to cell surface receptors, which trigger internalization of the nanoparticles into endosomes. A proportion of encapsulated materials escape the endosomes and are trafficked to the intracellular site of action (Reproduced with some modifications from Ref. 29, with permission from ©Science, and Allen and Cullis). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

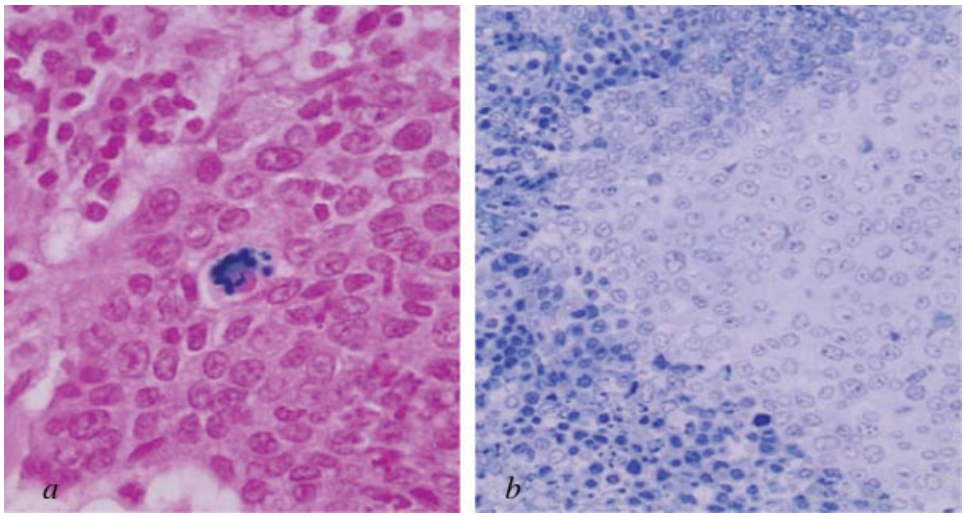

FIGURE 6 – Light micrographs. (*a*) Image of tumor tissue, with large tumor cell nuclei surrounded by non-tumorous small lymphocytes; positive iron staining of incorporated ferrofluids in tumor tissue (blue). (*b*) Serial section of the same tissue as in (*a*), ultrathin section ready for electron microscopy (Reproduced from Ref. 32, with permission from ©Springer and Alexiou *et al.*). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

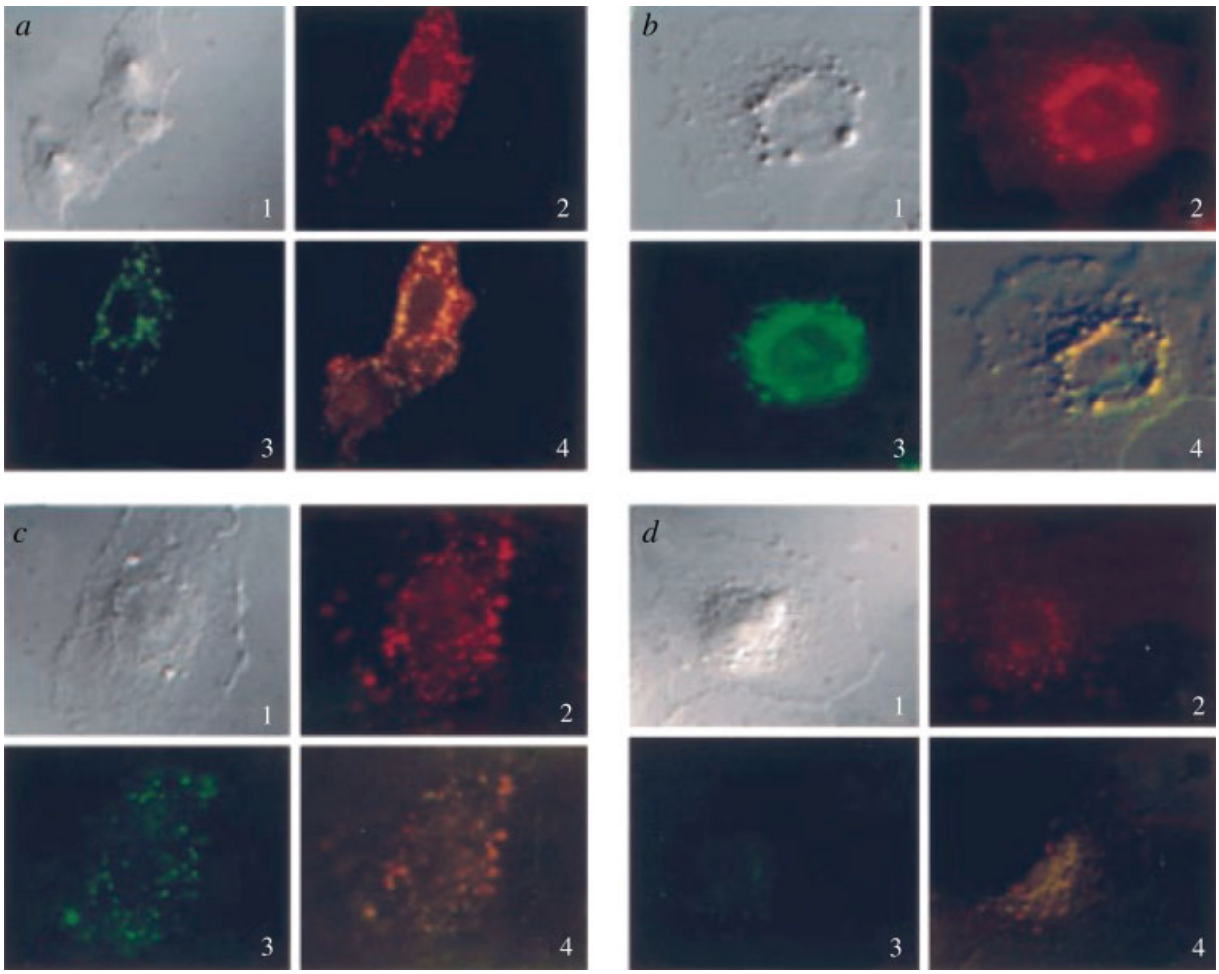

FIGURE 7 – Intracellular trafficking of Rh-PE-labeled and FITC-dextran-loaded TATp-liposomes within BT20 cells (human breast adenocarcinoma cells). Typical patterns of intracellular localization and integrity of TATp-liposome after 1 (*a*), 2 (*b*), 4 (*c*), and 9 h (*d*). 1, DIC light; 2, DIC with an Rh filter; 3, DIC with an FITC filter; 4, DIC composite of 1–3. Magnification: ×400 (Reproduced from Ref. 52, with permission from ©PNAS, and Torchilin *et al.*). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

membrane antigen protein expressed on the surface of prostate epithelial cells and are taken up by these cells resulting in a significantly enhanced *in vivo* cellular toxicity, thus killing cancer cells.[41,42]

Recently, the research of Alexiou *et al.* showed that magnetic drug targeting employing nanoparticles as carriers is a promising system for cancer treatment that does not cause side effects commonly observed in conventional chemotherapy.[32] They used iron

oxide nanoparticles covered with starch derivatives with phosphate groups that bind to mitoxantrone, a chemotherapeutical agent. It was demonstrated that a strong-magnetic-field gradient at a tumor site induces the accumulation of nanoparticles, and ferrofluids can become abundant in tumor tissue and tumor cells (Fig. 6). A dendrimer is another attractive candidate as a carrier of drugs for delivery to cancer cells because it has many unique characteristics.[43,44] Kukowska-Latallo *et al*. have synthesized folate-conjugated dendrimer nanoparticles coupled to methotrexate.[44] These nanoparticles accumulate in human KB tumors and livers tissue over 4 days after their administration because the liver and KB tumor cells express high levels of the folate receptor. They also studied the internalization of these nanoparticles into tumor cells. Their research demonstrated that this targeted dendrimer nanoparticles show a high antitumor activity and a marked toxicity.

Targeted nucleic acid delivery to cancer with nanoparticles

The development of efficient molecular medicines, including gene therapeutics, RNA therapeutics, and DNA vaccines, depends on an efficient means of transfer of DNA or RNA into a cell.[45,46] Gene therapy will have a wide variety of new medical applications when appropriate gene vectors are available. Viral vectors are widely used because the transfection efficiency is significantly high, but the safety of gene therapy using viral vectors is the most critical concern. There is a risk of developing severe immunological responses within a targeted host or tissue for adenovirus, as well as insertional mutagenesis for retroviruses when viruses are used as transfection vectors. Deaths have actually occurred in human trials, leading to the suspension of the use of viral vectors for gene transfection, which has been a major setback for this area of research.

Recently, controlled nonviral vectors have been developed.[47] Among nonviral vectors, nanoparticles as gene carriers have been extensively investigated, and also many exciting results have been obtained, particularly in cancer gene therapy, which demonstrated that nanoparticles as the gene carriers have a high potential for clinical applications in cancer therapy.[48,49]

Micro or nanoliposomes and biodegradable nanoparticles are commonly used as gene delivery carriers.[50,51] Torchilin *et al*. found that nanoliposomes modified with transcriptional activator protein (TAT) peptide (TATp-liposomes) rapidly and efficiently translocated into the cell cytoplasm and subsequently migrated into the perinuclear zone.[52] Figure 7 shows that 200 nm rhodamine-labeled TATp-liposomes loaded with FITC-dextran rapidly translocate into human breast adenocarcinoma cells (BT20). They also further studied pEGFP gene transfection into mouse fibroblast cells (NIH/3T3) and rat cardiomyocyte cells (H9C2). Wartlick *et al*. investigated the delivery of antisense oligonucleotides into tumor cells by human serum albumin (HSA) nanoparticles.[53] HSA nanoparticles containing different antisense oligonucleotides (ASO) are efficiently taken up into several tumor cell lines. Confocal laser scanning microscopy revealed that nanoparticles cross-linked with low amounts of glutaraldehyde, rapidly degraded intracellularly, leading to a significant accumulation of ASO in cytosolic compartments of tumor cells (Fig. 8). In 2004, a new system using ligand-targeted nanoparticles adapted for siRNA was synthesized.[54] Nanoparticles with siRNA are constructed using polyethyleneimine that is PEGylated with an Arg-Gly-Asp (RGD) peptide ligand attached at the distal end of polyethylene glycol (PEG), as a means to target the tumor neovasculature-expressing integrins and used to deliver siRNA that inhibits vascular endothelial growth factor receptor-2 (VEGF R2) expression and thereby tumor angiogenesis. After intravenous injection, these nanoparticles can be selectively taken up by tumor cells, and tumor growth rate and VEGF R2 expression level remarkably low.

Gold nanoparticles after peptide modification are also used for HeLa human cervical epithelial cells and HepG2 human hepatocarcinoma cells.[55] Their studies demonstrated that cellular translocation and nuclear targeting using synthetic cellular targeting peptides complexed to 20 nm gold particles depend on the attached peptide sequence. Figure 9 shows the cellular uptake of a nuclear localization signal peptide derived from the HIV Tat protein on gold nanoparticles. Recently, silica nanoparticles are attractive carriers for gene transfection to cancer cells, because the silica nanoparticle-DNA complex has a high transfection efficiency and a low toxicity.[48,56,57] The latest research focused in this review is that of Prasad's group in the State University of New York. They used organically modified silica nanoparticles as carriers of DNA into KB cells (human epithelia cancer cells) as a nonviral approach (Fig. 10).[58] Plasmids bound to amino-functionalized silica nanoparticles were completely protected against enzymatic digestion. Their research aroused much interest with respect to the treatment of brain diseases, such as encephaloma, neurodegenerative brain diseases. Other scientists claimed that biological-functionalized nanoparticles can cut the tumors' supply lines by destroying the tumors' vessels. During vascular remodeling and angiogenesis, endothelial cells show increased expression level of several cell surface molecules that potentiate cell invasion and proliferation.[37,59–61] One such molecule is integrin $\alpha_v\beta_3$, which plays a key role in endothelial cell survival during angiogenesis *in vivo*. Hood *et al*. showed that a cationic nanoparticle coupled to an integrin $\alpha_v\beta_3$-targeting ligand can deliver genes selectively to angiogenic blood vessels in tumor-bearing mice.[49] The therapeutic efficacy of this approach was tested by generating cationic nanoparticles conjugated to a mutant *Raf* gene, ATP^{μ}-*Raf*, which blocks endothelial signaling and angiogenesis in response to multiple growth factors. A systemic injection of cationic nanoparticles into mice resulted in the apoptosis of tumor-associated endothelial cells, ultimately leading to tumor cell apoptosis and a sustained regression of established primary and metastatic tumors (Fig. 11). This work was also highly regarded by other researchers and scientists.[62] Vascular targeting is promising for the delivery of drugs/

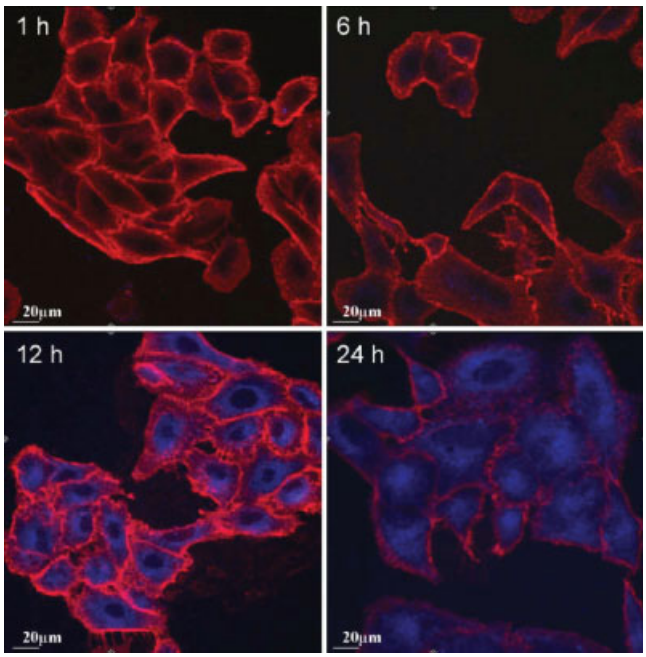

FIGURE 8 – Time-dependent cellular uptake and drug release of Cy5-PTO-loaded HAS nanoparticles. Autofluorescent HSA nanoparticles (green) were loaded with Cy5-PTO (blue), and incubated at 1 μM PTO concentration with A549 cells, and visualized by confocal microscopy (CLSM) using 40× magnification at time intervals of 1, 6, 12, and 24 hr. Cell membranes were stained with Alexak594-conjugated concanavalin A (red) (Reproduced from Ref. 53, with permission from ©Elsevier, and Wartlick *et al*.). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

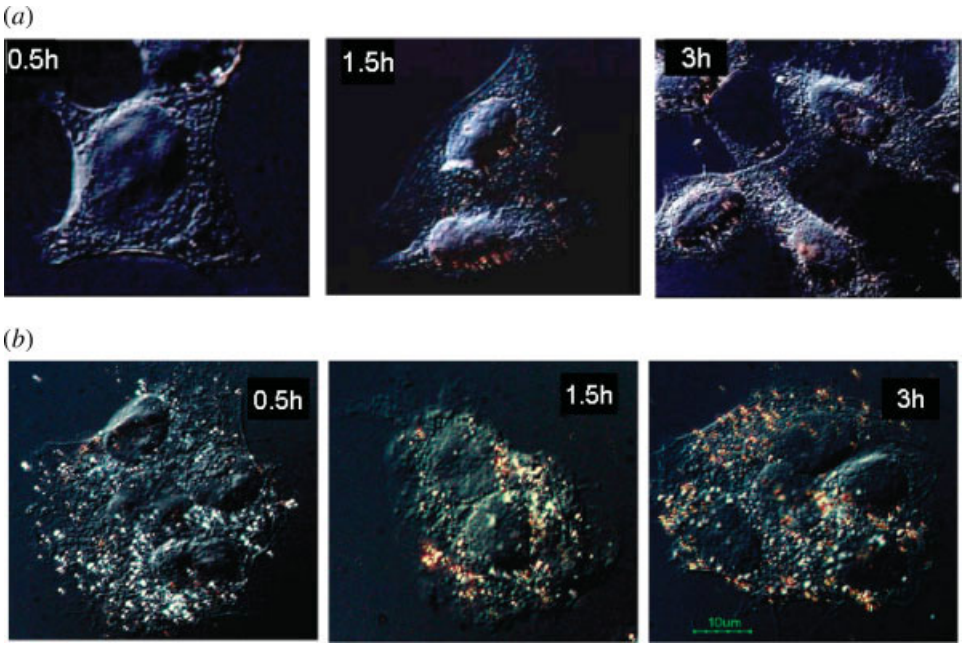

FIGURE 9 – Incubation of HeLa cells (*a*) and HepG2 cells (*b*) with 20-nm-diameter gold nanoparticles and observed by video-enhanced color differential interference contrast microscopy. The gold nanoparticles carried a nuclear localization signal peptide derived from the HIV Tat protein (Reproduced from Ref. 55, with permission from ©ASC Publications, and Tkachenko *et al.*). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

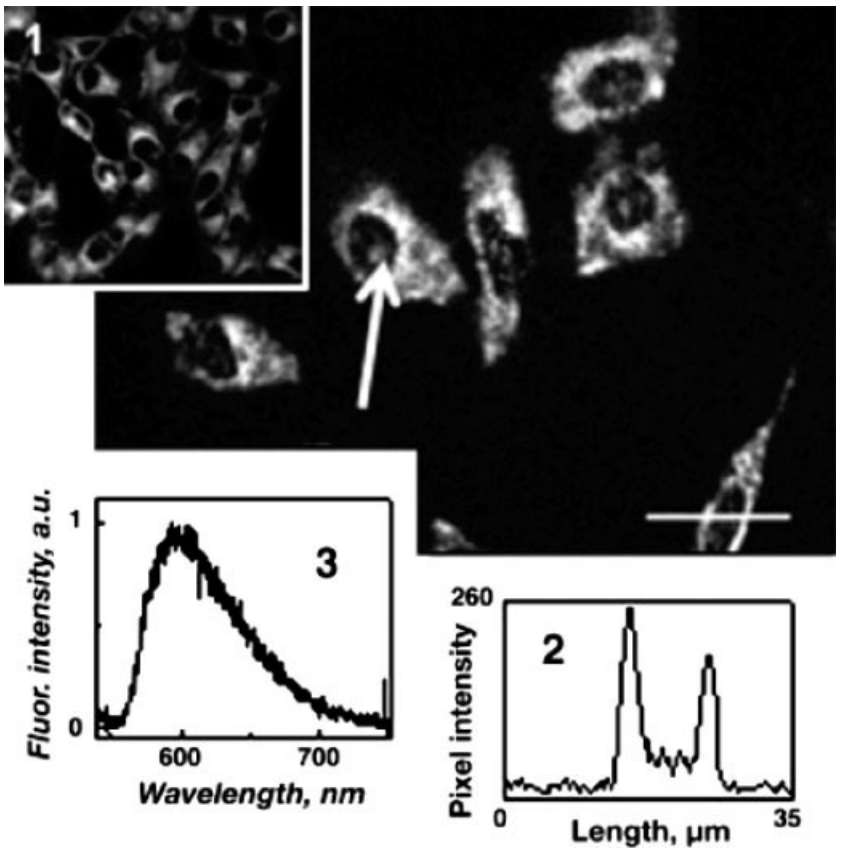

FIGURE 10 – Confocal fluorescence image of KB cells (human epithelial cancer cells) treated with organically modified silica nanoparticles previously incubated with DNA labeled with fluorescent dye EMA. The arrow indicates the area (nucleus) in which the fluorescence spectrum was taken; fluorescence intensity distribution was measured along the selected line. Inset: Confocal fluorescence image of KB cells treated with hydrophobic dye HPPH encapsulated in ORMN20 particles. Lower Right: Distribution of DNA-EMA fluorescence intensity along the selected line. Lower Left: Fluorescence spectrum from the cell nucleus (Reproduced from Ref. 58, with permission from ©PNAS, and Roy *et al.*).

gene therapeutics for cancer treatment. Recently, selectin expression on endothelial cell surfaces has been found to be another promising target in anticancer gene/drug therapy.[36] Although initial milestones were achieved in the above-mentioned researches, the extension of the applications of drug delivery systems remain a big problem.

In various transfection protocols, although a portion of DNA is liberated into the cytoplasm, the endocytic route of internalization of nonviral vectors with subsequent degradation of the delivered DNA by lysosomal nucleases poses another problem. An ideal vector should be efficient and nontoxic and should bypass the endocytic pathway to minimize DNA degradation. If the cancer

cell gene transfection efficiency is sufficiently high, gene therapy for human clinical applications would be realized.

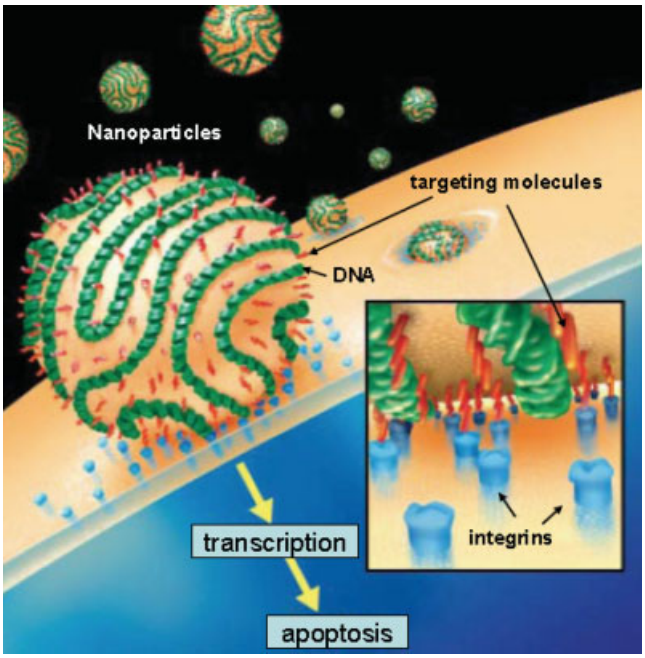

FIGURE 11 – Nanoparticles packed with targeting molecules (red) anchor to integrins (blue) outside of a tumor blood vessel cell before shuttling mutant DNA (green) into the cells. Integrins, such as $\alpha_v\beta_3$, are receptors and are highly expressed in the endothelium of tumor blood vessel, so the nanoparticles will target molecules to the tumor site where the molecules will accumulate, because the nanoparticle surfaces have been designed with specific ligands. The insert is the magnification of the contact interaction between nanoparticle and the cell surface (Reproduced with some modifications from Ref. 62, with permission from ©Science, and Couzin). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Cancer molecular imaging using nanoparticles/nanoprobes

Hybrid organic or inorganic nanoparticles are considered to have the potential as novel intravascular probes for diagnostics, for example, molecular imaging, cell or DNA labeling, immunohistochemistry, and tumor vessel imaging.[63–66] For this potential to be realized, the ability to target nanoparticles to specific tissues and cell types is important. Presently, there are several types of nanoparticle used in molecular imaging in cancer diagnosis, such as liposomes, dye-molecule-doped silica nanoparticles, Qdots, gold nanoparticles, immunotargeted nanoshells, perfluorocarbon nanoparticles, nanoshells, and magnetic nanocrystals.[67–74] Fluorescence labeling techniques have been used extensively in both biological research and clinical diagnosis. To achieve sensitive detection, there is an increasing demand for fluorescent labeling probes that are more intense, stable, and sensitive. Fluorophores commonly used, such as FITC, are not photostable and have a low fluorescence intensity, particularly in the case of real-time and long-term imaging observation or detection, these dye probes cannot fulfill higher demands in diagnosis. In 1999, Makarova *et al*.[70] successfully synthesized FITC-doped silica nanoparticles. This nanoparticle probe has a high potential in biological labeling.[70] Tan and coworkers in the University of Florida has synthesized a new form of highly luminescent and photostable nanoparticles, which were generated by doping a fluorescent dye (Rubpy) inside silica nanoparticles.[75] Because thousands of fluorescent dye molecules are encapsulated in silica matrix that protects Rubpy from photodamaging oxidation, the Rubpy-doped nanoparticles are extremely bright and photostable. They also used these nanoparticles successfully in various fluorescence labeling techniques, including fluorescence-linked immunosorbent assay, immunocytochemistry, immunohistochemistry, DNA microarray analysis, and protein microarray assay. By combining the high-intensity luminescent nanoparticles with the specificity of antibody-mediated recognition, ultrasensitive target detection has been achieved. This technique can be used to detect tumor antigen markers and provide some helpful information in early cancerization detection. On the other hand, the use of nanoliposomes in cancer diagnosis and imaging was reported.[76] Antibody-conjugated paramagnetic liposomes (diameter 300–350 nm) are used to visualize tumor angiogenesis *in vivo* by magnetic resonance imaging (MRI).[76] These nanoparticles with binding specificity for the $\alpha_v\beta_3$ receptor are

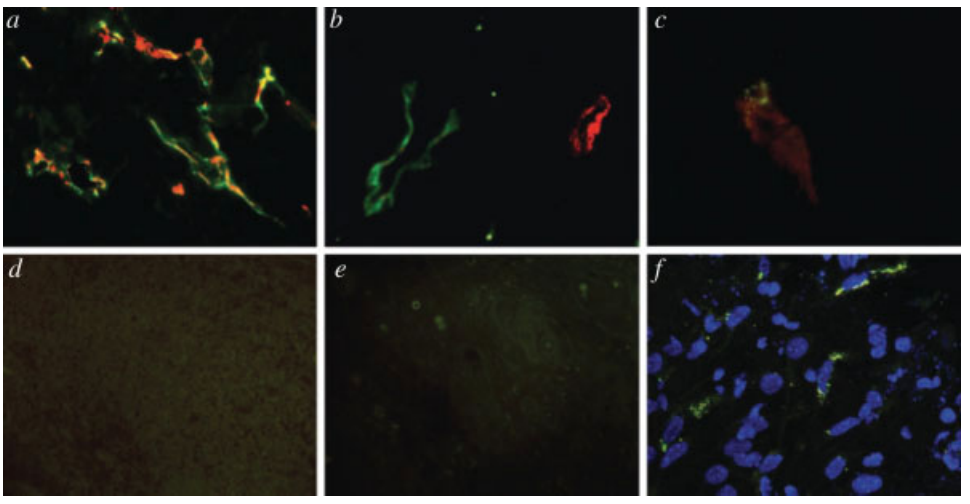

FIGURE 12 – *In vivo*-targeting of Qdots to the tumor vasculature is specific. Red F3 or LyP-1 Qdots, both PEG-coated, were injected into the tail vein of nude mice bearing breast carcinoma MDA-MB-435 xenograft tumors. Blood vessels were visualized by coinjecting tomato lectin (green). (*a*) F3 Qdots colocalize with blood vessels in tumor tissue. (*b*) LyP-1 Qdots also accumulate in tumor tissue, but do not colocalize with the blood vessel marker. (*c*) Red F3 Qdots and green LyP-1 Qdots injected into the same tumor-bearing mouse target different structures in tumor tissue. (*d*) GFE Qdots that bind to normal lung endothelial injected into tumor mice are not detected in tumor tissue. (*e*) F3 Qdots injected into tumor mice do not appear in the skin taken from an area next to the tumor. (*f*) LyP-1 Qdots are internalized by cells in tumor tissue (Reproduced from Ref. 66, with permission from ©PNAS, and Åkerman). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

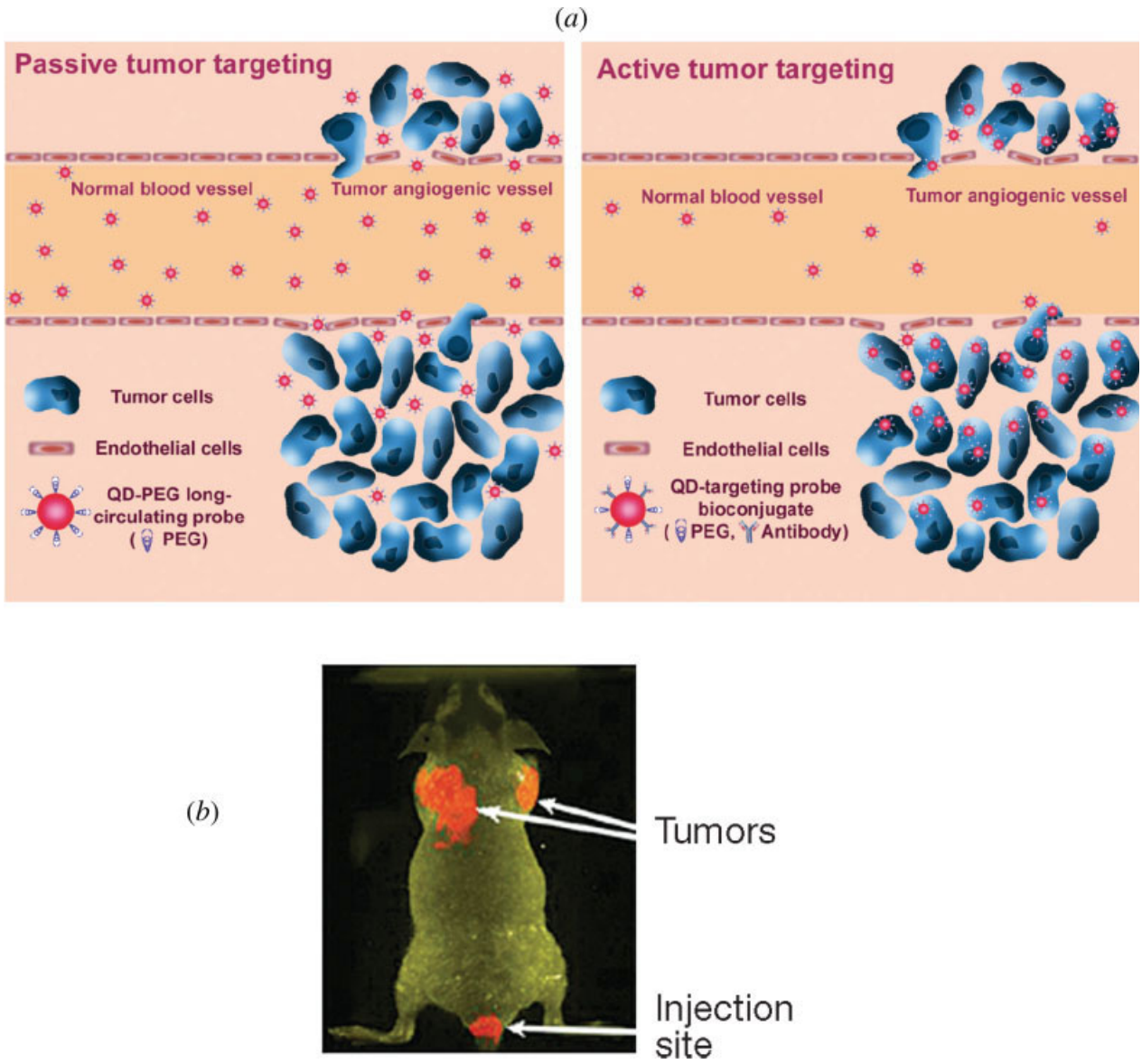

FIGURE 13 – Schematic illustration of bioconjugated Qdots for *in vivo* cancer targeting and imaging. (*a*) Permeation and retention of Qdot probes via leaky tumor vasculatures (passive targeting) and high affinity binding of Qdot-antibody conjugates to tumor antigens (active targeting). (*b*) *In vivo* fluorescence images of tumor-bearing mice using Qdot probes with anti-PEG-PSMA antibody conjugates surface modifications in mouse cancer model (PSMA: Prostate specific membrane antigen). *In vivo* fluorescence imaging was carried out using a macroillumination system. True-color fluorescence images were obtained using dielectric long-pass filters and a digital color camera (Reproduced from Ref. 79, with permission from ©Nature, and Gao). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

constructed by conjugating biotinylated antibodies against $\alpha_v\beta_3$ to the surface of liposomes via avidin linker proteins. The nanoliposomes are intravenously injected into the rabbit model of squamous cell carcinoma. The tumor vessel architecture is clearly visualized by MRI. On the basis of the above findings, perfluorocarbon nanoparticles are also designed to target $\alpha_v\beta_3$ receptors highly expressed on endothelial cells of the tumor neovascular system for MRI.[54,73]

One of the rapidly developing and most exciting interfaces of nanotechnology is Qdots in biology and medicine.[68,77,78] Qdots' properties of interest to biologists include high quantum yield, broad absorption with narrow and symmetric photoluminescence spectra, the unparalleled ability to size-tune fluorescence emission as a function of core size, high resistance to photobleaching, and exceptional resistance to photo- and chemical degradation.[68] In comparison with *in vitro* applications, *in vivo* applications of Qdots are more complicated and challenging. The selective targeting of peptide-coated ZnS-capped CdSe Qdots to mouse tumor vasculature cells *in vivo* was reported (Fig. 12), which will provide a new approach to tumor vasculature imaging, and can be used for monitoring the cancer treatment. Qdots may also be useful for tracking cancer cells *in vivo* during metastasis.[65,68,79,80] Multifunctional Qdot probes linked to tumor-targeting antibodies have been developed.[79] *In vivo* studies of mice expressing human cancer showed that these Qdot probes accumulate at tumor sites (Fig. 13). The ability to track cells *in vivo* without continuously sacrificing animals represents a substantial improvement over current techniques. However, the high toxicity of cadmium remains a major problem that needs to be solved before Qdots can be used in clinical setting. Despite this toxicity issue for Qdots, Qdots has a high potential in long-term real time imaging

in vivo, such as, intracellular traffick imaging, molecular interaction imaging, cellular imaging for basic cancer research.[81–84]

Magnetic nanocrystals have also shown great potential in molecular imaging of tumors by MRI. Recently, by a ''one-pot'' reaction process, Hu *et al*. have successfully generated the biocompatible Fe_3O_4 nanocrystals by covalently modifying Fe_3O_4 nanocrystals with monocarboxyl-terminated poly(ethylene glycol) (PEG) via its carboxylic group.[85] The nanocrystals are biofunctional and are modified to conjugate with a cancer-targeting anticarcinoembryonic antigen monoclonal antibody rch 24. These nanocrystals have satisfactory blood circulation time, and can be used in magnetic resonance detection of cancer *in vivo*.

Recently, superparamagnetic iron oxide nanoparticles (SPION) are also attractive, which were introduced as contrast agents shortly after the use of gadolinium chelates, and currently appear to be the preferred material.[86] The interest on SPION labeling is mainly because of the many excellent properties of SPION[1]: they provide the most change in signal (albeit hypointensity) per unit of metal[2]; they are composed of biodegradable iron, which is biocompatible and can thus be reused/recycled via cells using normal biochemical pathways for iron metabolism[3]; their surface coating, usually dextran, allows straightforward chemical linkage of functional groups and ligands[4]; they can be easily detected by light and electron microscopy[5]; and they can be magnetically manipulated and their magnetic properties can be changed according to size, with the potential to reveal their structural (bound) conformation. Weissleder and coworkers investigated the use of SPION for noninvasive detection of lymph-node metastases in prostate cancer by MRI.[87] MRI with this superparamagnetic nanoparticles correctly identified all patients with nodal metastases, and a node-by-node analysis had a significantly higher sensitivity than conventional MRI. Many excellent data were obtained in their experiments, and the imaging protocols used in this study have been adopted by most other MRI centers. Most recently, this group also developed another new X-ray-computed tomography imaging agent based on bismuth sulfide nanoparticles.[88] This polymer-coated Bi_2S_3 nanoparticles have a long circulation time and a high X-ray absorption, and can enhance imaging of vasculature. It is expected to be more applicable in the clinical monitoring and detection of cancer after multifunctionalization of the nanoparticle surface.

Although nanomedicine has provided some new breakthroughs for cancer therapy and diagnosis, the potential adverse human health effects resulting from exposure to novel nanomaterials should also be a concern. The biodistribution and movement of nanoparticles through tissues, and the phagocytosis, opsonization, and endocytosis of nanoparticles are all likely to affect on the potential toxicity of nanoparticles.[89] Recently, the new term of ''nanotoxicology'' has been often used by nanobiologists and pharmacologists.[90] For several nanoparticles, oxidative stress-related inflammatory reactions have been observed.[91] Presently, to better understand the potentially harmful side effects of nanomaterials, many nanobiologists call for an extensive investigation of nanotoxicological issues.[90,92,93] For instance, Qdots are good nanoprobes and are useful for *in vivo* biomedical imaging of cancer in animal models, but they are highly toxic because cadmium is a heavy metal; therefore, Qdots pose a high risk to human health.[94] On the other hand, although targeted nanoparticles have low toxicity to nontargeted tissue, there are still many reports that nanoparticles cause some toxicities in the liver, spleen, kidneys, lymph nodes, heart, lungs, and bone marrow.[95–97] Some nanoparticles are readily transported throughout the body. They are deposited in those organs, penetrate cell membranes, lodge in mitochondria, and may trigger injurious responses. To minimize the risks posed by nanomaterials, there are two basic avenues. One is to develop new highly biocompatible nanomaterials with low toxicity, such as, PLGA and silica nanoparticles. The other is surface modification of nanoparticles with biocompatible chemicals, such as polyethylene glycol and chitosan. Many great efforts are being made to develop nanoparticles satisfactory for clinic applications.

Concluding remarks

Nanomedicine is still at its early stages of development; however, its development is multi-directional and rapid. The flexibility to modify and adapt nanoparticles to meet the needs of pathological conditions of cancer or tumor either for drug delivery in therapeutic applications or as a diagnostic tool (targeted molecular imaging) is one of the important characteristics of this technology with newly discovered cancer-specific targets. Although some great achievements have been attained, many challenges still remain. For example, the low encapsulation efficiency for some drugs also need to be overcome; some nanoparticles are still toxic for clinic application. On the other hand, due to the complexity of the body and the layers of barriers, the efficiency of targeting nanoparticle accumulation to the tumor is not very high and the targeting is not perfect. Effective targeting would require a dual-focus strategy, a better understanding of the target and a simultaneous development of the targeting system. Only some bottlenecks have been addressed, the full *in vivo* potential of nanoparticles in targeted imaging and drug delivery to cancer or tumor can be realized, and nanoparticles will have wide clinical applications.

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