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Nanotechnology platforms and physiological challenges for cancer therapeutics

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Abstract Nanotechnology is considered to be an emerging, disruptive technology that will have significant impact in all industrial sectors and across-the-board applications in cancer research. There has been tremendous investment in this area and an explosion of research and development efforts in recent years, particularly in the area of cancer research. At the National Institutes of Health, nanomedicine is one of the priority areas under its Roadmap Initiatives. Moreover, in 2005 the National Cancer Institute alone committed $144.3 million over 5 years for its Alliance for Nanotechnology in Cancer program. Much research and development is progressing in the areas of cancer diagnostics, devices, biosensors, and microfluidics, but this review will focus on therapeutics. Current nanotechnology platforms for cancer therapeutics encompass a vast array of nanomaterials and nanodevices. This review will focus on six of the most prominent and most widely studied: nanoshells, carbon nanotubes, dendrimers, quantum dots, superparamagnetic nanoparticles, and liposomes. All of these nanotechnology platforms can be multifunctional, so they are frequently touted as "smart" or "intelligent." This review will discuss the shared approaches in the design and development of these nanotechnology platforms that bestow such characteristics to the nanoparticles. Finally, the review will raise awareness of the physiological challenges for the application of these therapeutic nanotechnologies, in light of some recent advances in our understanding of tumor biology.

Key words: Nanoparticles; Nanotechnology platforms; Cancer therapeutics; Nanotherapeutics

Nanotechnology platforms for cancer therapeutics

Nanoshells

Nanoshells are nanoparticle beads that consist of a silica core coated with a thin gold shell [1]. Manipulation of the thickness of the core and the outer shell permits these beads to be designed to absorb and scatter specific wavelengths of light across the visible and near-infrared (NIR) spectrum. Their primary application is in thermal ablation therapy by exploiting their ability to absorb light. Meanwhile, their ability to scatter light has potential for cancer imaging. The most useful nanoshells are those that have a silica core diameter of ~120 nm with a 10-nm layer of gold shell, because these strongly absorb NIR light (~800 nm) and can create intense heat that is lethal to cells. This NIR light can penetrate several centimeters of human tissue without causing harm, because tissue chromophores do not absorb much energy in the NIR range [2].

Loo et al have shown that antibodies can be attached to nanoshells to get them to specifically recognize and target cancer cells (e.g., breast adenocarcinoma cells overexpressing human epidermal growth factor receptor-2) in vitro [1]. The antibodies were first attached to polyethylene glycol (PEG), and this antibody-PEG complex was then attached to the nanoshell surface through a sulfur-containing group located at the distal end of the PEG linker. O'Neal et al have demonstrated the ability of intravenously administered nanoshells and NIR treatment to completely eliminate tumors by thermal ablation in vivo [3]. Thermal therapies

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using lasers have been used for some time, but simple heating cannot discriminate between tumors and the surrounding healthy tissue. Thus, the energy source harms the intervening and surrounding healthy tissue even when focused beams are used. The benefit of the nanoshell-mediated approach is that that the energy can pass through the healthy tissue and leave the neighboring cells intact, while killing only the tumor cells that have been targeted by the nanoshells.

Carbon nanotubes

Carbon nanotubes are a distinct molecular form of carbon atoms that was discovered in the late 1980s. There has been tremendous enthusiasm over carbon nanotube applications in many industrial sectors, in part because they have been actively promoted as possessing the advantages of being 100 times stronger than steel with only one-sixth of its weight, and with unusual heat and conductivity properties. In the area of cancer therapeutics, carbon nanotubes have primarily been used for transporting DNA cargoes into the cell and for thermal ablation therapy, in much the same way as the nanoshells described above. Kam et al have shown that single-walled carbon nanotubes 1 to 2 nm in diameter and carrying a cargo of 15-mer DNA adsorbed onto their surfaces can be internalized by cells and accumulate in the cytoplasm without causing cytotoxicity [4]. At 4°C, there was minimal cellular uptake of DNA-carrying carbon nanotubes, suggesting an energy-dependent uptake mechanism. Exposing the DNA-nanotube containing cells to several 10-second pulses of NIR caused endosomal rupture, unloading of the DNA from the carbon nanotubes, and translocation into the nucleus. Again, the cells showed normal morphology and no apparent death under these conditions.

Carbon nanotubes can also be used for targeted thermal ablation therapy. Like nanoshells, carbon nanotubes can absorb NIR light to generate intense heat. For example, continuous irradiation with NIR (808-nm laser at 1.4 W/cm^2) for 2 minutes will heat up a 25 mg/L solution of single-walled carbon nanotubes to 70°C and lead to boiling of the solution with longer exposures [4]. Kam et al have shown that folic acid can be adsorbed onto the carbon nanotubes to allow specific binding to cancer cells that overexpress folate receptors and subsequent receptor-mediated endocytosis. Tumor cells that had internalized the folic acid–bound carbon nanotubes were selectively destroyed upon irradiation with NIR, whereas receptor-free normal cells that had not internalized these carbon nanotubes were not harmed by NIR irradiation. The localization of carbon nanotubes, and whether or not they were internalized by cells, could be visualized by attaching fluorescent tags to the carbon nanotubes.

Recently, Z. Zhang et al have demonstrated that carbon nanotubes carrying short (or small) interfering RNA (siRNA) can rapidly enter tumor cells, then release the siRNA to exert RNA interference on target gene expression [5]. They have shown that the delivery of siRNA via carbon nanotubes into tumor cells not only silenced the target gene (i.e., reduced both its mRNA and protein levels), but also inhibited the proliferation of cancer cells in vitro and suppressed tumor growth in mouse models, upon intralesional injection of siRNA-conjugated carbon nanotubes. The siRNA was coupled to single-walled carbon nanotubes that had been specially synthesized to contain -CONH-$(CH_2)_6$-NH_3^+ functional groups. According to the authors, this positive charge functionalization mediates the conjugation of siRNA to the carbon nanotubes. They used siRNA that specifically targeted murine telomerase reverse transcriptase. Telomerase is the key enzyme that stabilizes chromosomes by adding TTAGGG repeats to the telomere ends, and telomerase reverse transcriptase is its catalytic subunit. The activation of telomerase is critical for immortalization, and it is detected in a majority of malignant tumors but not in most normal somatic cells. Hence, inhibition of telomerase activity is actively pursued in targeted cancer therapy. Z. Zhang et al also demonstrated that by 48 hours after treatment, the cells treated with siRNA-nanotubes showed morphological features associated with senescence and reduced telomerase reverse transcriptase activity. Past efforts to deliver siRNA to target cells have often been thwarted by the instability of siRNA and low efficiency of uptake. The use of carbon nanotubes as a vehicle for delivery of siRNA presents great promise.

Dendrimers

Dendrimers are spherical polymers that are normally less than 5 nm in diameter. Their key useful feature is the polymer branches that provide vast amounts of surface area to which therapeutic agents and targeting molecules could be attached. The prototypical dendrimer starts with an ammonia (NH_3) core that is reacted with acrylic acid to produce a tri-acid molecule. This molecule is then reacted with ethylenediamine to produce a tri-amine, and this is known as generation 0 (G0) product. This tri-amine is reacted with acrylic acid to produce a hexa-acid, then reacted with ethylenediamine to produce a hexa-amine (G1), and so on. This alternation of reaction with acrylic acid then with ethylenediamine continues until the desired generation is reached. Sugars or other molecules can also be used as the starting core, so long as they have multiple, identical reaction sites. Thus, it is possible to create a surface consisting of multiple amines or multiple acids, and these two kinds of surfaces provide the means of attaching different functional components.

In early 2006, Majoros et al synthesized and characterized a multifunctional dendrimer conjugated with fluorescein isothiocyanate (for imaging), folic acid (for targeting cancer cells overexpressing folate receptors), and paclitaxel (chemotherapeutic drug) [6]. They synthesized from an ethylenediamine core a G5 poly(amidoamine) dendrimer whose primary amino groups on the surface were first neutralized through partial acetylation to provide enhanced

solubility of the dendrimer and prevent nonspecific targeting interactions during delivery. The three types of functional molecules were conjugated to the remaining nonacetylated primary amino groups. Fluorescein was attached through a thiourea bond; folic acid was covalently conjugated via condensation between the γ-carboxyl group of the folic acid and the primary amino group of the dendrimer; and paclitaxel was attached covalently through an ester bond, which is characterized by ease of cleavage through enzymatic hydrolysis. This dendrimer conjugate would be considered a prodrug that remains inactive until cleavage of the drug from the carrier. Finally, the remaining primary amino groups were converted to -OH to prevent nonspecific targeting during delivery.

This group demonstrated in vitro that drug-free dendrimer conjugates were not cytotoxic even though they bound to the cells, and that drug-loaded dendrimer conjugates had no effect on folate receptor–negative cells. Approximately 100 nM of dendrimer conjugates were necessary to see drug susceptibility of folate receptor–positive cells, and the toxicity observed was due to intracellular delivery of paclitaxel and not merely due to its presence in the media. At 200 nM (equivalent to 800 nM of free paclitaxel), the dendrimer conjugates were toxic to both folate receptor–positive and folate receptor–negative cells as a result of nonspecific binding. Drug-free dendrimer conjugates were not toxic to the cells at the concentration of 200 nM.

The first study to demonstrate successful in vivo–targeted drug delivery to cancer cells by intravenously administered nanoparticles involved methotrexate-carrying dendrimers that could recognize cells expressing folate receptors [7]. Targeted delivery of methotrexate via dendrimers was shown to be markedly more effective at delaying the growth of epithelial cancer xenografts in mice than the drug given alone. In addition to methotrexate and folic acid, these dendrimers also carried fluorescein to permit tracking of their location in the bloodstream.

Quantum dots

Quantum dots are frequently referred to as nanocrystals in the lay press, although the term "nanocrystals" is not restricted to quantum dots. They range from 2 to 10 nm in diameter and are made of semiconductors, the most common being cadmium selenide capped by zinc sulfide (CdSe/ZnS). Quantum dots are composed of 10–50 atoms, and they confine electron-hole pairs to a discrete quantized energy level. When excited with ultraviolet light, they fluoresce in different neon colors depending on their size, which determines the energy level of the quantum dot. Larger particles emit light in the red end of the visible spectrum, whereas smaller particles emit in the blue range.

When quantum dots were first developed some 20 years ago for electronics and optics, no one realized their potential for application in biomedicine. However, their use as research tools has expanded markedly in the last few years, and they are currently being used as probes for high-resolution molecular imaging of cellular components and for tracking a cell's activities and movements inside the body. Quantum dots can be also be attached to various proteins and receptors to monitor with which molecules they interact and in what part of the cell they are found. For example, they can be linked to antibodies for the detection of cancer markers such as human epidermal growth factor receptor-2 and other antigens on the cell surface [8]. Tumor cells labeled with quantum dots can be used to track metastasis to specific tissues and organs [9]. The greatest advantage of using quantum dots over radioactive tags or organic fluorophores such as fluorescein or cyanine dyes is that quantum dots can fluoresce for several months in a living animal [10], they do not degrade or bleed through, and they are much more resistant to photobleaching.

Most recent advances have attempted to use quantum dots as carriers for siRNA, similar to the use of carbon nanotubes in that capacity mentioned above. In the study by Tan et al, siRNA targeting the gene encoding human epidermal growth factor receptor-2 was conjugated to quantum dots, which not only functioned as the carrier but also permitted monitoring of the transfection efficiency [11]. Human epidermal growth factor receptor-2 antibodies attached to the quantum dots permitted targeted delivery of the siRNA–quantum dots to breast cancer cells overexpressing this receptor, and subsequent receptor-mediated endocytosis of the quantum dot conjugates. Gene-silencing effect of the conjugated siRNA was determined by enzyme-linked immunosorbent assay and demonstrated that siRNA transported into cells via quantum dots can achieve desirable silencing effects on the target gene through RNA interference.

Because cells are impermeable to quantum dots, they must be coated with special molecules or antibodies to facilitate their uptake by cells. This property can be exploited to devise a method that uses extracellular enzymes to modulate cellular uptake of quantum dots. Y. Zhang et al have recently demonstrated this proof of concept by conjugating to quantum dots special peptide ligands consisting of (1) a "transporter" segment required for transport into cells, (2) a "blocker" segment whose presence inhibits cellular uptake, and (3) a "linker" between the transporter and blocker that could be cleaved by a specific enzyme [12]. The "transporter" was a peptide made up of arginine residues. The authors demonstrated that oligomers of four to nine arginine residues conjugated to quantum dots facilitated their uptake by cells, whereas shorter arginine oligomers were not effective. One of the enzymes selected for the study was matrix metalloprotease-2 (MMP-2), which is a secreted endopeptidase crucial for degradation of the extracellular matrix (ECM). Because this enzyme is needed for malignant tumor cells to breach the ECM, its overexpression correlates with advanced tumor stage and increased invasion and metastasis. The peptide ligand was R4XPLGVRGE4: four cationic arginine residues (transporter), connected to the substrate for MMP-2 (amino acid

sequence PLGVR), connected to four anionic glutamate residues (blocker). X represents 6-aminohexanoyl, a spacer inserted to minimize unfavorable interactions with the enzyme. They showed in vitro that polycationic peptide-mediated uptake of quantum dot conjugates was blocked by the presence of negatively charged groups on the quantum dots, and that their cellular uptake occurred only in the presence of MMP-2, which removed the negatively charged groups and left R4XPLG still attached to the quantum dots. This strategy was just as successful with MMP-7 (using a different peptide ligand).

Because semiconductors are poisonous heavy metals, toxicity is a huge obstacle to clinical application of quantum dots for humans. Currently, their application is restricted to in vitro or animal studies, and researchers are actively trying to develop different ways to coat them so that they would be safe for use in people.

Superparamagnetic nanoparticles

Superparamagnetic nanoparticles refer to iron oxide particles or magnetite (Fe_3O_4) particles that are less than 10 nm in diameter. They have been around for years as contrasting agents for magnetic resonance imaging (MRI). Many groups have explored the use of magnetic fields to localize magnetic nanoparticles to targeted sites, a system known as magnetic drug targeting. As with other nanoparticles, functionalization of these superparamagnetic nanoparticles are getting functionalized so as to permit specific tumor targeting. Iron oxide nanoparticles can be water-solubilized with hydrophilic polymer coatings, such as dextran or PEG. In fact, attaching PEG to nanoparticles in general, not just to iron oxide particles, is a well-documented means of sterically preventing opsonization of nanoparticles in the serum and reducing their uptake by the reticuloendothelial system. This effectively enhances biocompatibility and increases the circulation time of nanoparticles [13]. Iron oxide nanoparticles can also be made hydrophobic by coating with aliphatic surfactants or liposomes (resulting in magnetoliposomes) [14].

Magnetic nanoparticles can be remotely activated using electromagnetic fields, and they can also be used to thermally treat cancers [15]. Under the influence of an alternating field, superparamagnetic nanoparticles undergo Brownian relaxation, in which heat is generated by the rotation of particles in the field. However, concentrations of 0.01% to 0.1% iron oxide are necessary to raise the tissue to critical temperatures for thermal ablation, and these concentrations are hard to achieve via intravenous administration. Most recently, superparamagnetic nanoparticles have been used in clinical thermotherapy of locally recurrent prostate cancer [16]. Thermotherapy is defined as the ability to attain at least hyperthermic temperatures of up to 42°C, which can render cancer cells more susceptible to the effects of radiation and cause some apoptosis [16]. Iron oxide nanoparticles in water (known as magnetic fluid; 112 mg/mL concentration) were injected transperineally into the prostate, and an alternating magnetic field was applied. Because of the very low clearance rate of these nanoparticles from the tumor mass, serial thermotherapy treatments can follow a single magnetic fluid injection, and the patients received six thermotherapy treatments of 60 minutes duration at weekly intervals. The iron oxide nanoparticles in tissue specimens were detected using computed tomography. MRI was not suitable because of signal void in the areas of high concentrations of iron oxide nanoparticles. Maximum temperature of 55°C could be achieved in the prostate. The median temperature in 90% of the prostates was 40.1°C, and the median thermal dose was 7.8 cumulative equivalent minutes at 43°C. The aim of this clinical study was to demonstrate that magnetic nanoparticle–mediated heating is feasible and that hyperthermic to thermoablative temperatures can be achieved in the prostate tissue. However, magnetic nanoparticle–based thermotherapy or thermoablation has yet to be refined for monotherapy, and in the foreseeable future their efficacy would most likely be in combination therapy.

Liposomes

Liposomes are vesicles made up of a lipid bilayer, resembling tiny cells with a cell membrane but nothing in the core. Research on using liposomes to encapsulate and deliver chemotherapeutics has been performed since the late 1970s, and in the early 1990s they were extensively studied as potential vectors for gene therapy. At the time no one referred to them as "nanoparticles," but liposome research has gained considerable renewed momentum in association with the nanotechnology movement. Liposomes do not constitute novel nanotechnology, and their sizes, ranging from 90 to 150 nm, are slightly bigger than what would qualify as nanotechnology according to the conventional definition (i.e., having a dimension of $\leq$100 nm), but a significant portion of what is considered as nanotechnology research in biomedicine today is represented by liposome research.

There are many different type of lipids with different head groups, different fatty acid chain lengths, and different melting temperatures (T_m). Hence, by manipulating the formulation of liposomes, they can be constructed to be temperature or pH sensitive to permit controlled release of their contents [17]. For example, Mills and Needham have constructed temperature-sensitive liposomes that can release the drug contents in tens of seconds at clinically attainable hyperthermia (39–42°C) [18]. Administration of these liposomes loaded with doxorubicin, in combination with local hyperthermia, resulted in complete regressions of human tumor xenografts in all of the mice studied [19].

Mills and Needham were able to create such liposomes by incorporating monopalmitoylphosphatidylcholine (MPPC) or monostearoylphosphatidylcholine (MSPC) into dipalmitoylphosphatidylcholine (DPPC) bilayer membrane to support the formation of lysolipid-stabilized pores in the membrane that facilitated the release of contents [20]. Pure DPPC liposomes are able to release only 20% of their

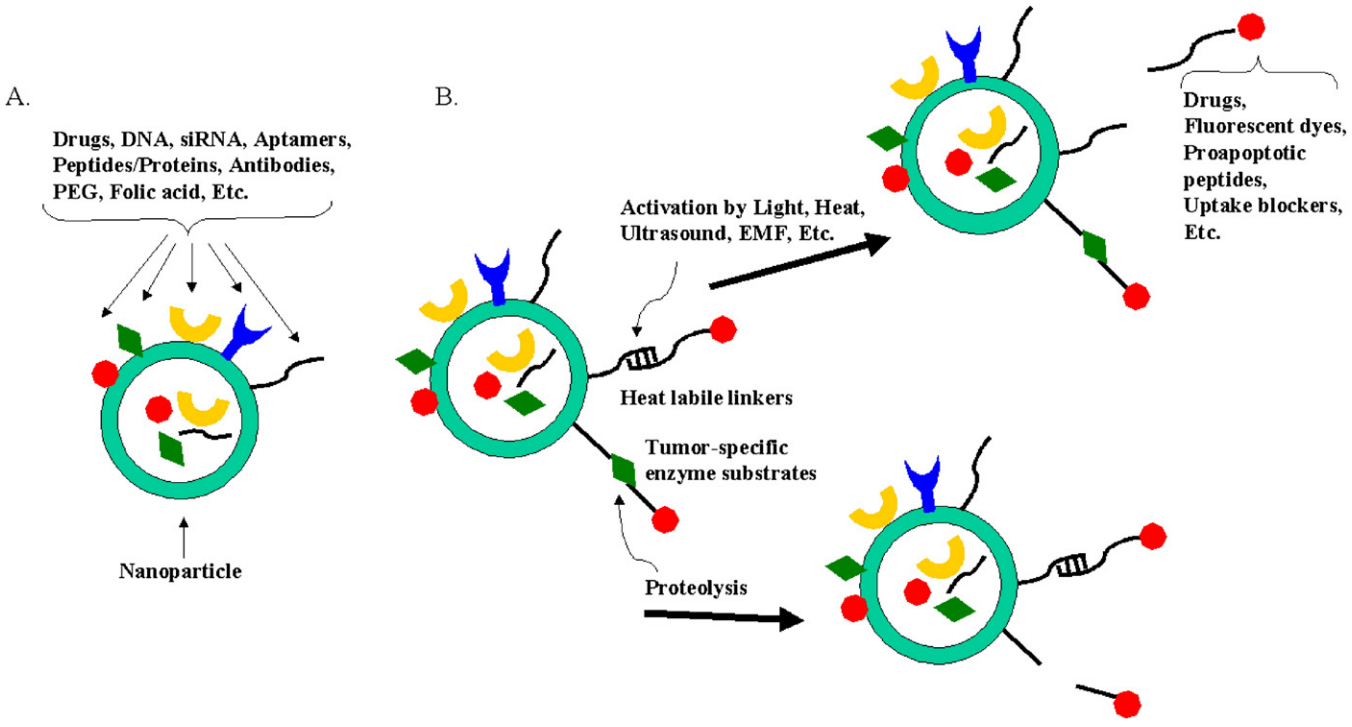

Fig 1. Multifunctionality schemes. **A,** Simple scheme. **B,** Complex scheme.

content even after 5 minutes of incubation at their T_m of 42°C, but incorporating lysolipid in DPPC bilayer membrane can markedly enhance content release (both the amount and the speed of release) as the membrane liquefies. During phase transition there is anomalous membrane permeability to drugs and other small molecules that are encapsulated within the liposome. As the membrane passes through its phase transition from solid to liquid phase, leaky interface regions develop at boundaries between still solid lipid domains and melting, liquid lipid domains. In addition, large incompatibilities in molecular packing and hydrophobic matching characterize the lipids at these interface regions [21]. This permeability at the phase transition is enhanced by the inclusion in the bilayer of a second lipid, which creates additional mismatches and lipid heterogeneity, and further disturbs molecular packing [22].

Although clinical application of the nanoparticles described above as cancer therapeutics has yet to be realized, liposome nanoparticles are already on the market. A prime example is Doxil (doxorubicin hydrochloride in liposome) for ovarian cancer.

Common approaches to generating multifunctionality

The strategies for generating multifunctional nanoparticles share common approaches, whether the nanoparticles are nanoshells, carbon nanotubes, dendrimers, iron oxides, quantum dots, liposomes, or other nanoparticles. In addition to these platform nanoparticles, there are a large variety of nanoparticles constructed of other types of materials. They all involve encapsulation, covalent conjugation, or noncovalent adsorption of various moieties (e.g., chemicals, drugs, DNA, small interfering RNAs, peptides, aptamers, ligands, stealth molecules, homing molecules, and other cell-targeting molecules) to allow the nanoparticles to recognize and locate the tumor, deliver a load or kill the tumor cells, and permit visualization and imaging (see Figure 1, *A*). Different peptides that can act together synergistically could be strategically attached in combination, and the nanoparticles could also be loaded with multidrug regimens.

Engineering these "smart" nanoparticles could involve even more complex schemes for targeted drug release or nanoparticle activation, by using heat-labile or protease-susceptible tethers (see Figure 1, *B*). The heat-labile linkers could be a variety of molecules, including DNA with heat-labile hydrogen bonding between complementary strands. Substrates for tumor-specific or tumor environment–specific enzymes could be chosen to serve as the protease-susceptible linkers. For example, Harris et al have developed a strategy for superparamagnetic nanoparticle self-assembly by designing biotin and neutravidin-coated iron oxide nanoparticles that are inhibited from self-assembly by PEG chains that are anchored to the nanoparticles via matrix MMP-2–cleavable peptide substrates [23]. Only upon proteolytic removal of surface PEG through MMP-2 cleavage of the peptides can the nanoparticles self-assemble through unhindered biotin-neutravidin interactions. MMP-2 is a tumor-specific protease correlated with cancer invasion and metastasis, and this assembly and clustering of nanoparticles permits MRI detection of tumor-derived cells that are producing the protease and enhanced image contrast of tumor invasion in the body.

Hence, researchers may exploit tumor-specific processes and environments (e.g., abnormal pH and O_2 levels; unique cell surface molecules and receptors; ECM remodeling and associated proteolytic enzymes overexpressed in tumor microenvironments) to trigger enzymatic activation of nanoparticles via bonds that are sensitive to degradation under these conditions, and cause drug release from nanoparticle surface or nanoparticle accumulation within tumors or in specific regions of the body. Although these are the general strategies, inducing the nanoparticles to actually perform in vivo as predicted by theory and addressing the biocompatibility, biostability, and biodistribution issues involve extensive research.

Challenges for delivery of nanoparticles

It is believed that localization and accumulation of nanoparticles preferentially in tumors may be achieved by enhanced permeability and retention of nanoparticles based on passive extravasation of particles <400 nm in most tumors [24-26]. This is attributed to the leakiness of tumor vessels caused by openings between defective endothelial cells, wide interendothelial junctions, incomplete or absent basement membrane, loosely attached or absent pericytes (cells that provide support for the endothelial cells), and large numbers of transendothelial channels or pores [26,27]. Whether it be through the physical enhanced permeability and retention effect or the use of specific targeting molecules, nanoparticles may successfully reach the tumors, but their ability to penetrate the tumor mass may be impaired because of barriers created by abnormal tumor physiology. Abnormal tumor structures, such as physically compromised vasculature, abnormal ECM, and high interstitial fluid pressure, can create constraints that thwart effective delivery of nanotherapeutics.

Normal vasculature is ~8–10 μm in diameter and uniformly structured. Tumor vasculature, on the other hand, has highly variable vessel diameter that ranges from 20 to 100 μm, and the vascular organization and branching pattern are highly chaotic. Moreover, the blood flow is erratic in tumor vessels; the flow is intermittent, periodically abating and even reversing directions. In some parts of the blood vessel blood rushes by very rapidly, whereas no blood flows through other parts. The blood vessel may be leaky along one side but not along the other side. Some segments of blood vessel may not be leaky at all, and proliferating cancer cells can cause intratumoral vessels to compress and collapse [28]. It is well recognized that the irregularity of the tumor vasculature with its abnormal blood flow and impaired venous and lymphatic drainage creates high interstitial fluid pressure, making the diffusion of nutrients and chemotherapeutics throughout the tumor very inefficient, and it may present challenges to the effective diffusion of nanoparticles as well [24].

There are also extravascular barriers to delivery, whereby nanoparticles can extravasate but cannot penetrate through the ECM of the tumors. Yuan et al measured the interstitial penetration of intravenously administered liposomes (90 nm in diameter) in human colon adenocarcinoma xenografts in mice and have shown that the liposomes were able to extravasate the tumor vasculature but remained within 10–20 μm away from the blood vessel [29]. Instead of distributing homogeneously, the liposomes formed perivascular clusters that did not move significantly and could be observed for as long as 1 week. Intratumoral injection of adenovirus nanoparticles (100 nm in diameter) resulted in the transfection of the tumor cells only along the needle track and did not diffuse readily across ECM [30]. It would take days for a particle having the size of a virus or liposome to traverse 100 μm of the tumor mass and months for it to traverse 1 mm. A smaller particle, such as an IgG molecule with a hydrodynamic radius of 5 nm, would require ~2–3 days to traverse 1 mm of the tumor mass. This inability to efficiently penetrate the tumor and affect cells distant from the vessels or the injection site may limit therapeutic efficacy of nanoparticles.

Recently, McKee et al have shown that fibrillar collagen restricts nanoparticle distribution [31]. The majority of replication-defective herpes simplex virus particles (150 nm in diameter) injected intratumorally into human melanoma xenografts in mice were located only in collagen-poor areas and could not penetrate the collagen matrix. The experiment was repeated with comparably sized, quantum dot–encoded, silica spheres that were also ~150 nm but lacked the ability to bind to ECM proteins, and similar results were observed in which they were also excluded from the collagen. Dextran particles with diameter of 40 nm and IgG molecules were able to penetrate into the collagen-rich regions and distribute relatively uniformly within the tumor, indicating that this collagen exclusion was particle dependent. Netti et al have shown that when the tumor is treated with collagenase the diffusion of nanoparticles across the ECM increases by 100% [32]. It has also been shown that when collagenase is administered with virus particles into the tumors, the area of particle distribution is greater and the therapeutic effect of the virus is improved as demonstrated by tumor regression [31].

What Jain has recently proposed is a “normalization” hypothesis that calls for administration of antiangiogenic agents to remodel and normalize the existing tumor vasculature [33]. Because impaired blood supply and interstitial hypertension interfere with the delivery of therapeutics to solid tumors, the goal is to transiently “normalize” the abnormal structure and function of tumor vasculature to restore efficient blood flow within the tumor, decrease the high interstitial fluid pressure characteristic of tumors, and improve the delivery of therapeutics, including nanotherapeutics. If the tumor vasculature is made more efficient for delivery of nanoparticles, the efficacy of these nanoparticles and therapeutic outcome would be enhanced accordingly. Morphological changes reflecting normalization include decrease in tumor vessel diameters to more

normal diameter size of ~10 μm, decrease in vascular permeability to high-molecular-weight molecules, decrease in tumor hypoxia, and decrease in interstitial fluid pressure. This is essentially a novel paradigm for combination therapy. Molecules that have been shown to be successful at normalization and are candidates for combination therapy include Herceptin (trastuzumab), which is a monoclonal antibody specific to human epidermal growth factor receptor-2 used to treat metastatic breast cancer and has also been shown to decrease amounts of vascular endothelial growth factor (VEGF) [34], and Avastin (bevacizumab), which is an monoclonal antibody specific to VEGF [35].

Summary

In hospitals and clinics, the current approaches for cancer treatment are still limited to surgical resection, radiation, and chemotherapy. These are highly invasive or nonspecific, and often accompanied by side effects and toxicity to healthy cells. The promises of nanotechnology in cancer research lie in the potential to overcome these drawbacks. As in the case of photothermal ablation or activation by electromagnetic fields, nanoparticle therapy can be remotely controlled by external source of energy and can be minimally invasive. Nanotherapeutics are often multifunctional, in which a single molecule can permit detection, diagnosis, imaging, transport and controlled release of cargo, and cell destruction. This is because many of the nanoparticles can be functionalized with several different types of molecules simultaneously—DNA, RNA, targeting molecules and peptides, carbohydrates, and imaging agents. Nanoparticles can selectively target cancer biomarkers and cancer cells, allowing greater efficacy of lower doses of drugs, more sensitive diagnosis, early detection requiring minimal amount of tissue, monitoring of the progress of therapy and tumor burden over time, and destruction of solely the cancer cells. Hence, they are touted as being “smart” and “intelligent.”

However, there is nothing intrinsic about nanoparticles that allows them to specifically target tumor cells and distinguish them from the normal cells, or to distinguish among multiple cell types, resulting in minimal damage to healthy tissues. It is by design that they acquire the ability to recognize unique surface signatures of tumor cells. Moreover, developing clever strategies and knowing which molecules to attach to the nanoparticles require knowledge of tumor-specific receptors that would allow endocytosis of nanoparticles, tumor-specific biomarkers that facilitate identification of cancers, tissue-specific and tumor-specific homing proteins, and tumor-specific enzymes that can permit selective uptake into cells or accumulation in tumor microenvironments. In summary, basic knowledge of cell biology, tumor biology, immunology, and cancer biology is essential to the rational design of nanoparticles for cancer therapeutics, and advancement in nanotechnology will be critically dependent on the advancements made in cancer biology.

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