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Minireview

Molecules and Cells

Nanomaterials for Cancer Therapy and Imaging

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A variety of organic and inorganic nanomaterials with dimensions below several hundred nanometers are recently emerging as promising tools for cancer therapeutic and diagnostic applications due to their unique characteristics of passive tumor targeting. A wide range of nanomedicine platforms such as polymeric micelles, liposomes, dendrimers, and polymeric nanoparticles have been extensively explored for targeted delivery of anti-cancer agents, because they can accumulate in the solid tumor site via leaky tumor vascular structures, thereby selectively delivering therapeutic payloads into the desired tumor tissue. In recent years, nanoscale delivery vehicles for small interfering RNA (siRNA) have been also developed as effective therapeutic approaches to treat cancer. Furthermore, rationally designed multi-functional surface modification of these nanomaterials with cancer targeting moieties, protective polymers, and imaging agents can lead to fabrication versatile theragnostic nanosystems that allow simultaneous cancer therapy and diagnosis. This review highlights the current state and future prospects of diverse biomedical nanomaterials for cancer therapy and imaging.

INTRODUCTION

Cancer is a group of diseases which cause an abnormal and uncontrolled cell division coupled with malignant behavior such as invasion and metastasis (Djojosubroto et al., 2003). Despite remarkable advances in modern medical sciences, cancer remains a disease difficult to treat and becomes a leading cause of death worldwide (around 13% of all deaths) (Heath and Davis, 2008). During the past 70 years, the number of cancer death has continued to rise, as compared to the slight increase in the number of people died of other diseases such as heart diseases, cerebrovascular diseases, and pneumonia. In these days, radiotherapy and chemotherapy are the principal treatment modalities aimed at eradicate solid tumors that are located deep inside the body. However, these methods have suffered from their non-specific mode of action, which not only kills cancer cells but also harms normal cells at the same time (Hirsch et al., 2003; Park et al., 2009). For example, the most common chemotherapeutic agents such as paclitaxel and doxorubicin exhibit anti-cancer effects by inducing apoptotic death of rapidly dividing cells, but they can also kill several types of normal cells that divide rapidly in ordinary circumstances (Spencer and Faulds, 1994; Yoo et al., 2000). Since the current chemotherapy is mainly based on a whole-body treatment with the chemotherapeutic agents, it is inevitable to cause many dangerous side effects associated with the non-selective cytotoxic effect of the medications.

Nanotechnology is a relatively new field of scientific research concerning the study of the structure, property, and behavior of materials below several hundred nanometers in size (Ferrari, 2005). In recent years, nanotechnology has attracted significant interests in cancer therapeutics because of its huge potential to offer an innovative paradigm to overcome the problems of existing chemotherapeutic agents. For example, a variety of nanovehicle platforms with sizes (10-200 nm) ideally favorable for endocytic intracellular uptake, high drug loading, and specific targeting to the tumor tissues can be rationally designed (Davis et al., 2008; Duncan, 2003). These artificially engineered nanomaterials will greatly improve therapeutic efficacy of the loaded chemotherapy drugs while reducing non-specific toxicity, thereby making it possible to achieve safe and effective cancer treatment. Furthermore, tremendous efforts have been recently devoted to develop multi-functional theragnostic nanosystems for both cancer diagnosis and therapy, which can deliver drugs specifically to tumors and simultaneously monitor their therapeutic response by visualizing tumor legions in the body (Nie et al., 2007; Salvador-Morales et al., 2009). In the near future, there is no doubt that the convergence of nanotechnology and biological sciences will revolutionize the entire discipline of cancer medicines. This review aims to introduce key concepts of cancer angiogenesis and tumor vasculatures, to highlight the distinguished advantages of nanoparticulate drug carriers and the molecular mechanisms underlying their selective cancer targeting effects, and to introduce several state-of-the-art examples of current nanomaterials for cancer diagnosis and therapy.

TUMOR PHYSIOLOGY AND TUMOR TARGETING PRINCIPLE USING NANOMATERIALS

Angiogenesis in cancers

Angiogenesis is the process involving the formation of new blood vessels from pre-existing vascular networks. Under normal circumstances, angiogenesis is observed during inflammation, tissue regeneration, and wound healing. Excessive or

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abnormal angiogenesis is commonly associated with many pathological diseases, including vascular malformations, atherosclerosis, obesity, arthritis, as well as cancer (Carmeliet and Jain, 2000; Fidler et al., 2010). Microvascular networks are absolutely essential for the development of solid tumors. For example, most tumors cannot grow further beyond a certain size (about 1-2 mm in diameter) without newly generated blood vessels due to inadequate blood supply (Folkman, 1974). As tumors grow rapidly, they soon encounter a hypoxic microenvironment deprived of oxygen and nutrients. This hypoxic condition initiates signaling events that trigger the up-regulation of multiple pro-angiogenic factors in the tumor lesion (Bergers and Benjamin, 2003; Kohandel, 2007). Among them, vascular endothelial growth factor (VEGF), also called vascular permeability factor (VPF), plays an important role in regulating the process of tumor angiogenesis. VEGF has been shown to stimulate the proliferation, migration, and invasion of endothelial cells by interacting with a family of tyrosine kinase receptors expressed on vascular endothelium (Ferrara and Davis-Smyth, 1997; Lee et al., 2007a). In addition, VEGF is known to have the ability to enhance the permeability of microvessels, favoring the rapid and reversible increases in extravasation of plasma proteins in tissue (Roberts and Palade, 1995; Senger, 1983). This process appears to be mainly associated with the inward migration of endothelial cells and fibroblasts from leaky blood vessels, resulting in the construction of new capillary vessels and mature stromal tissue near the hypoxic tumor lesions (Dvorak et al., 1995).

Unique characteristics of tumor vasculatures

The blood vessels found in solid tumors have abnormal physiological and morphological characteristics distinct from normal vasculatures (Jain, 2001; Ruoslahti, 2002). A better understanding of the characteristics of tumor vasculatures has encouraged the design and development of effective therapeutic approaches against cancers. An investigation into the tumor vasculatures revealed that tumors have a highly chaotic and irregular arrangement of blood vessels as compared to the vascular structures of normal tissues (Campbell, 2006; Morikawa et al., 2002). An overabundance of anionic phospholipids and proteoglycans is another characteristic of tumor vessels (Ran et al., 2002). In addition, the vascular networks of tumors have an increased permeability to circulating macromolecules and a significantly higher growth rate of endothelial cells in comparison with those in healthy tissues (Denekamp, 1984). The enhanced microvessel permeability is likely associated with the creation of abnormally opened inter-endothelial junctions. The size of vascular gap openings of tumors, usually falling within the range of 400 to 600 nm, is remarkably larger than that observed among the blood vessels in most normal tissues (Yuan et al., 1995). These findings suggest that the nanostructured materials of comparable size to the vascular pores can be exploited to penetrate into leaky tumor vasculatures and selectively deliver the loaded anti-cancer agents to the tumor tissues.

Distinguished advantages of nanomaterials as drug carrier

Progress in nanotechnology and medicine has led to an explosive development of a variety of nanostructured materials for therapeutic and diagnostic applications (Couvreur and Vauthier, 2006). Over the past decades, nanoscale drug delivery systems based on polymeric micelles, liposomes, and inorganic nanoparticles have been the subject of intensive research due to their therapeutic potential against cancers (Torchilin, 2005; Wagner et al., 2006). These nanomaterials provide many unique advantages that are not obtainable with other conventional anticancer therapies.

First, nanomaterials possess a small size similar to biological macromolecules such as peptides, proteins, and nucleic acids (Fig. 1). For scale, they are usually tens of nanometers in diameter, and approximately 100-1000 times smaller than the size of a single cancer cell. Due to the small size and dimensional similarities to biomolecules, nanomaterials exhibit a far greater intracellular uptake as compared with micron-sized particles, making them excellent candidates for cancer-targeted drug delivery (Goldberg et al., 2007). Furthermore, the internalized nanomaterials are able to interact with the biomolecules at specific intracellular compartments, possibly allowing for the manipulation of target signal pathways involved in cancer survival and proliferation (Tang et al., 2003). More importantly, nanomaterials also offer attractive possibilities to circumvent the vascular barriers and biological defense systems of the body. For instance, micro-sized particles, whose sizes are comparable to those of microbes, are easily taken up by the macrophages widely distributed throughout the body. This leads to rapid clearance of the microparticles from the bloodstream by the reticuloendothelial defence mechanism (Mok et al., 2009; Peer et al., 2007). Considering that extremely tiny capillaries that are about 2.3 micrometers in diameter have been observed (Potter and Groom, 1983), well-designed nanomaterials of controlled size range (sub-200 nm) can possibly gain access to many areas of the body via the circulating system, thereby increasing the opportunities to deliver drug payloads precisely to the tumors.

Second, nanomaterials can carry a large number of imaging and/or therapeutic agents owing to their enormous surface area relative to their total volume. For example, a polymeric nanoparticle having an average diameter of 70 nm is able to contain approximately 2,000 drug molecules (Bartlett and Davis, 2007), while a polymer-drug conjugate carries only nine drug molecules per molecule (Lee et al., 2008a). Such high drug loading capacity of nanomaterials is very advantageous for achieving significant therapeutic efficacy of cancer medicine. Additionally, they offer a possibility of surface modification with multiple targeting moieties (such as small molecules, peptides, or antibodies) for effective cancer targeting. Recent researches have proven that the multiple attachment of a targeting ligand significantly enhances the intracellular uptake of the ligand-functionalized nanovehicles in the target cancer cells via the multivalent binding to the cell-surface receptors (Hong et al., 2007; Montet et al., 2006). Hence a nanocarrier system possessing a number of targeting ligands can be effectively exploited for targeting to the tumor tissues with minimal non-specific uptake.

Lastly, nanomaterials hold great potential to overcome many limitations of the conventional chemotherapeutic agents. In general, most of chemotherapeutic agents are not readily dissolved in aqueous solution. Paclitaxel is a good example of water insoluble anti-cancer drugs. Paclitaxel has been extensively used in treating ovarian, breast, and other cancers due to their strong apoptotic effects on cancer cells (Lee et al., 2009a). However, the poor solubility of paclitaxel causes severe difficulties in preparing stable formulations for effective anticancer therapy (Bae et al., 2007; Yi et al., 1998). It is worth noting that nanovehicles such as polymeric micelles and liposomes are able to enhance water solubility of such hydrophobic anticancer drugs by stably incorporating them in the hydrophobic microenvironments (Hubbell, 2003). In addition, encapsulation in these nanovehicles improves their bioavailability and therapeutic efficacy in the bloodstream following systemic admini-

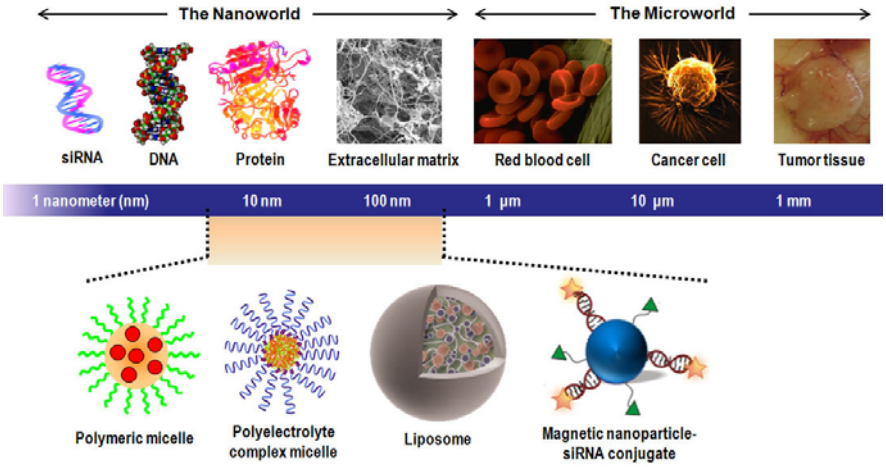

Fig. 1. A schematic diagram displaying several important biological systems and artificially engineered nanomaterials in a wide range of sizes.

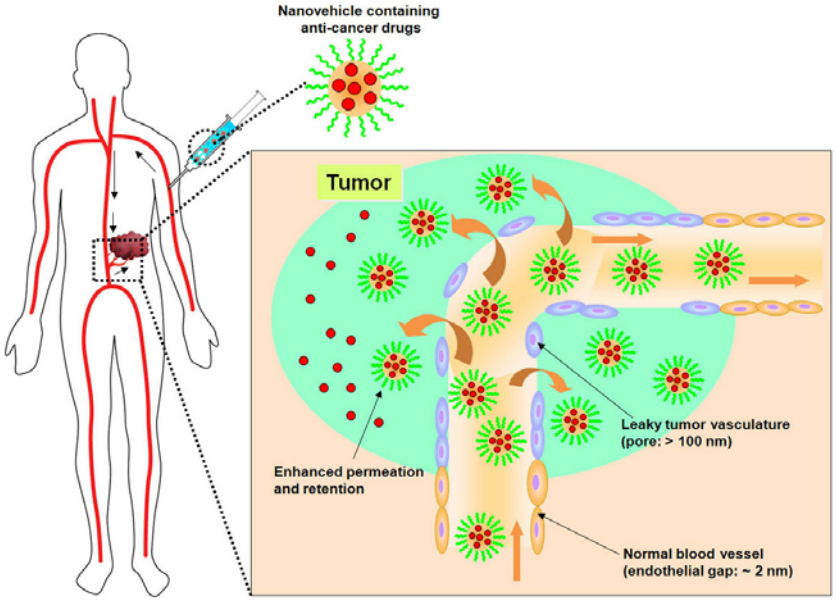

Fig. 2. Schematic illustration depicting the cancer-targeted accumulation of nanovehicles via the enhanced permeability and retention (EPR) effect.

stration. For example, traditional drugs with relatively low molecular weight are rapidly cleared by the kidney, which lowers the effective concentration in the target site (Allen and Cullis, 2004). Compared to these small molecule drugs, an administration of nanovehicle-incorporated drugs substantially increases the circulation time in the bloodstream so that sufficient amount of drugs can reach the target tissue. Another advantage of nanomaterials is their ability to provide high levels of control over drug toxicity and release profiles. Since the nanovehicles are able to stably encapsulate cytotoxic drugs in the interior, they will not only minimize toxicity of drugs to healthy tissues, but also protect them from premature metabolic degradation. Moreover, a new class of nanomaterial that releases their therapeutic payloads in response to external signals (e.g., light, temperature, pH, and tumor environments) has been recently studied (Lee et al., 2008b; Rivera Gil et al., 2010). These nanosystems are expected to further improve the therapeutic performance of the anti-cancer agents with enhanced delivery efficiencies in the tumor tissues.

Tumor targeting via enhanced permeability and retention effect

Macromolecules and nanoparticulate drug carriers such as polymeric micelles and liposomes preferentially extravasate from leaky tumor vasculatures and accumulate in tumors. This phenomenon now known as “enhanced permeability and retention (EPR) effect” has been the focus of researches for anti-cancer drug discovery and development (Matsumura and Maeda, 1986). Unlike the blood vessels found in most normal tissues, angiogenic tumor vessels usually have gaps larger than 100 nm between adjacent vascular endothelial cells (Yuan et al., 1995). Due to the presence of abnormally wide pores in tumor blood vessels, nanovehicles containing anti-cancer drugs can penetrate into the leaky tumor vasculatures, but not transport through tight inter-endothelial junctions in normal tissues (Fig. 2). Furthermore, the accumulated nanovehicles tend to be retained in the tumor tissues where no lymphatic drainage system is available for clearing macromolecules (Peer et al., 2007). As a result, they exhibit improved accumulation in the tumors than in normal tissues via the EPR effect and then release a large

amount of the loaded anti-cancer drugs specifically into the vicinity of the tumor cells, thereby allowing for effective anticancer therapy with minimum drug toxicity. Recent experiments have shown that diverse vascular mediators such as angiotensin II (AT-II), bradykinin, prostaglandin, and nitric oxide (NO) also induce significant augmentation of the EPR effect (Maeda, 2010). For instance, AT-II causes contraction of the smooth muscle layer surrounding the capillary vessels, which in turn elevates systemic blood pressure and increases blood flow volume in tumor tissues. This facilitates an enhanced extravasation of circulating nanovehicles into the tumor interstitium, consequently improving the tumor-selective delivery of anticancer agents.

NANOTECHNOLOGICAL APPROACHES FOR CANCER THERAPY AND IMAGING

Nanomaterials for targeted delivery of anti-cancer agents

Over the past decades, a variety of nanoscale drug delivery systems have been extensively explored to deliver anti-cancer agents specifically to cancers. These nanosystems include polymeric micelles, polyelectrolyte complex micelles, liposomes, dendrimers, nanoemulsions, and nanoparticles. Several representative examples of such nanosystems are illustrated in Fig. 1. Polymeric micelles are spherical and nanoscale (10-100 nm) colloidal carriers formed by the self-assembly of amphiphilic copolymers having both hydrophilic and hydrophobic segments in aqueous solution (Duncan, 2003). They have a unique core/shell nanostructure where the hydrophobic segments of the amphiphilic copolymers form an inner core surrounded by a layer of the hydrophilic segments. These polymer assemblies have attracted much attention as useful drug carriers for cancer therapy because they are able to incorporate water insoluble anti-cancer drugs, such as paclitaxel and camptothecin, within their hydrophobic core. In addition, the hydrophilic shell layer of these micelles can protect the incorporated drugs from degradation by enzymes, avoid non-selective uptake by macrophages distributed in the body, and hence allow for targeting cancerous tissues via the EPR effect (Kataoka et al., 2001). Despite these desirable properties, there is a need for further improving the *in vivo* stability of polymeric micelles. Since their structures are primarily retained by noncovalent interactions, polymeric micelles tend to dissociate in the bloodstream and be subsequently excreted through the kidney (Burt et al., 1999). In order to overcome such limitation, many technological approaches have been used to enhance the stability of polymeric micelles by chemically cross-linking in the inner core domain or within the outer shell layer (Kim and Park, 2002).

Polyelectrolyte complex micelles have recently emerged as an alternative class of polymeric micelles for efficient delivery of charged therapeutic agents (Park et al., 2006). They are usually produced by electrostatic interactions between anionic macromolecules and the di-block copolymers composed of cationic segments and hydrophilic segments. The polyelectrolyte complex micelles generally possess a spherical core/shell architecture where the oppositely charged molecules generate a charge-neutralized inner core and the hydrophilic segments form an outer shell layer exposed to aqueous solution. In contrast to the polymeric micelles, the polyelectrolyte complex micelles can be extensively applied for intravenous and intracellular delivery of various bioactive macromolecules including peptides, proteins, and nucleic acids (Mao et al., 2006). Moreover, they can be designed with different structures and compositions, making them applicable to a wide range of biopharmaceutical applications (Ferrari, 2005; Oh and Park, 2009). In recent years, a number of targeting ligands (e.g. folic acid, transferrin, peptides, or antibodies) have been covalently coupled onto the surface of the micelles for achieving effective tumor targeting and penetration (Davis et al., 2008).

Liposomes are spherical self-closed structures comprised of one or more of concentric lipid bilayers (Juliano and Stamp, 1975). They have an inner aqueous compartment enclosed by those lipid bilayers which are similar to the structures of biological membranes. Liposomes provide many distinctive advantages as drug delivery vehicles. First, they can carry hydrophilic therapeutic agents into their aqueous interior and also water insoluble anti-cancer drugs into the hydrophobic domain within the lipid bilayers. They also have relatively high drug loading capacity so that tens of thousands of drug molecules can be entrapped in their structures (Allen and Cullis, 2004). In addition, liposomes are generally considered to be biocompatible and known to cause very little antigenic, allergic, and toxic reactions because they are usually composed of naturally-derived phospholipids (Torchilin, 2005). Furthermore, they are able to efficiently deliver the drug payloads to cells or even into intracellular compartments (Chen et al., 1996). These unique properties make liposomes excellent candidates to deliver therapeutic or diagnostic agents to tumor tissues. However, liposomes tend to be recognized and cleared by macrophages in the reticuloendothelial system due to their relatively large size (50-400 nm). Hence many attempts have been made aiming to enhance *in vivo* stability of liposomes by incorporating polyethylene glycol (PEG) onto their surface. It has been shown previously that PEG-stabilized liposomes exhibit a prolonged plasma half-life compared to unmodified ones because the surface-exposed PEG chains form a protective layer around liposomes to reduce clearance by the reticuloendothelial defence mechanism (Klibanov et al., 1991; Mok et al., 2009). Recent efforts have been directed towards combining the long-circulating liposomes with targeting moieties for effective tumor-selective drug delivery.

siRNA and RNA interference

The discovery of RNA interference (RNAi) in mammalian cells has introduced a novel therapeutic tool for treatment of human diseases (Elbashir et al., 2001; Meister and Tuschl, 2004). RNAi is triggered by double-strand RNA precursors that are processed into short RNAs 19 to 23 nucleotides in length which recognize its complementary messenger RNA (mRNA) for its subsequent degradation (de Fougerolles et al., 2007; Dykxhoorn and Lieberman, 2006). Since the short double-stranded RNAs, or small-interfering RNA (siRNA) can modulate gene expression post-transcriptionally and sequence-specifically, they have been widely utilized for discovering and validating drug targets, transgenic studies, and development of RNA-based drugs with transient action (Dykxhoorn and Lieberman, 2006). Especially, siRNA therapeutics has drawn great attention due to their wide application potentials for treating the various kinds of human cancer by minimally invasive routes (Oh and Park, 2009; Reischl and Zimmer, 2009). siRNAs targeting tumor-related genes such as Bcl-2 and survivin, and angiogenic factors such as VEGF have been shown to inhibit tumor growth (Holle et al., 2004; Kim et al., 2006a; Miao et al., 2007).

siRNA nanomedicine for cancer therapy

The utilization of siRNA as cancer therapeutics remains a great challenge due to its unfavorable characteristics for delivery. The first biological barrier of siRNA delivery is its susceptibility to nucleases which causes its rapid degradation when administered into the body, resulting in short circulation time in the blood for its therapeutic effects (Whitehead et al., 2009). The

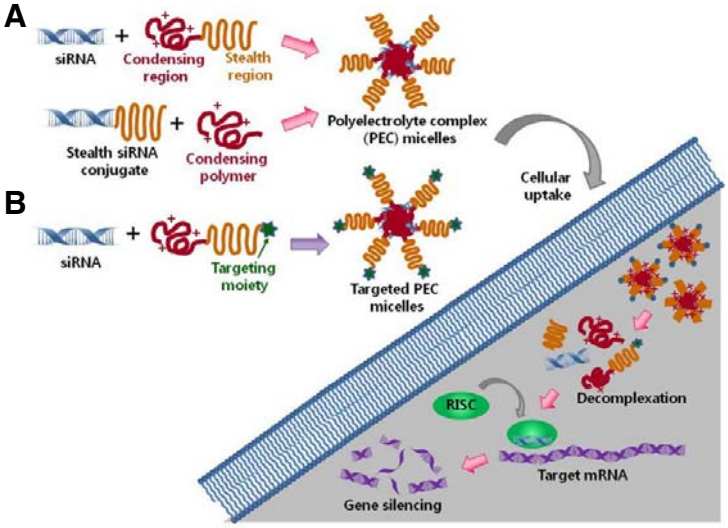

Fig. 3. siRNA delivery by polyelectrolyte complex (PEC) micelles. (A) siRNA is condensed with a cationic material by electrostatic interactions into nano-sized PEC micelles. A stealth region is introduced to improve stability and prolong blood circulation *in vivo*. (B) A targeting moiety can be incorporated to the PEC micelles for localization to target sites.

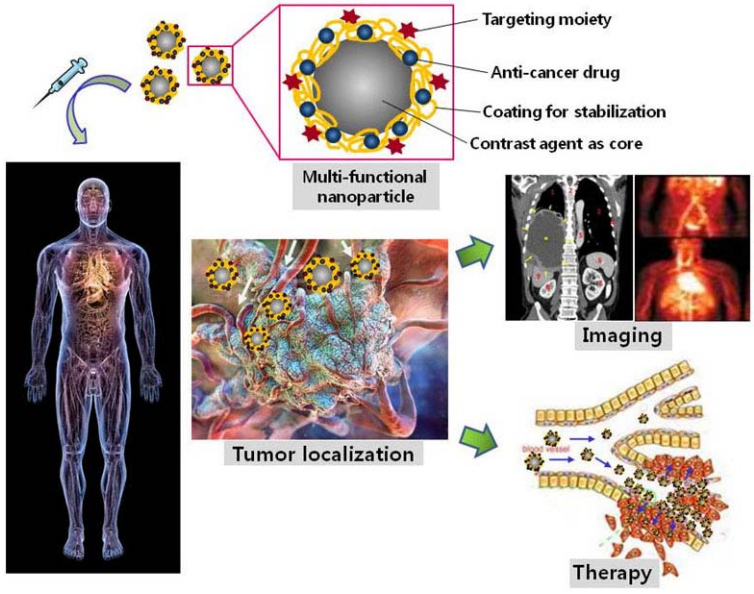

Fig. 4. Theragnostic nanomaterials: a contrast agent material carrying anti-cancer drug is localized into the tumor site of interest, which can be monitored *in vivo* by imaging techniques.

next challenge is to deliver the siRNA into the cells for its desired functions. However, the negative charge of the oligonucleotides results in its weak interaction with the cellular membrane and low internalization into the target cells (Whitehead et al., 2009). The final major challenge is to allow the internalized siRNA to escape the endosome to prevent its degradation and perform its biological functions in gene silencing (Oh and Park, 2009). To overcome these hurdles, siRNA has been incorporated into various types of nanoparticulate formulations including liposomes, polyelectrolyte complexes, polymeric nanoparticles, and inorganic nanoparticles (Dobson, 2006; Park et al., 2006; Schroeder et al., 2009). The most widely used methodology to deliver nucleic acid drugs is to condense the negatively charged molecules using cationic materials, made of polymers, lipids, and peptides, into nano-sized complexes (Dalby et al., 2004; Höbel et al., 2010; Mok and Park, 2008). The nanocomplexed particles containing the siRNA are then delivered to the target cells more efficiently due to the physical protection of the RNA to the host's systems. In addition, the neutral to positive charges and the higher stiffness of the particles enhance cellular recognition of the siRNA and subsequent internalization into cells. Conventional carrier materials such as lipofectamine, polyethylenimine (PEI), polyarginine, and polyhistidine, have been reported as efficient carriers. The siRNA-containing particles are either uptaken into the cells by transcytosis or endocytosis. In the case of endocytosed particles, another advantage of most of these cationic carriers is that they allow the siRNA-containing particles to escape from the endosome by the proton sponge effect (Akinc et al., 2005).

Another strategy to further prolong the bioavailability of the siRNA is to introduce a stealth molecule to the system. Polyethylene glycol (PEG) has been the most widely used polymer to produce anti-fouling surfaces for biomedical nanomaterials to prevent recognition by the host's defense system and its rapid clearance. Previous studies have utilized PEG for siRNA delivery by conjugating it to cationic polymers, such as poly(L-lysine) (Meyer et al., 2008) or polyethylenimine (PEI) (Mao et al., 2006) as carriers. The resultant nanosystems include a PEG shell as

the stealth region, and a core with the complexed cationic polymer containing the siRNA drug (Fig. 3A). An alternative strategy is to introduce the stealth region to the siRNA by conjugation, which is subsequently complexed with the cationic material (Jung et al., 2010; Kim et al., 2006a; 2008a). Delivery systems that localize the siRNA at target sites have also been developed by introducing targeting moieties to the condensing material (Fig. 3B). Bioactive molecules such as the TAT peptide (Kang et al., 2005), folate (Kim et al., 2006b), and hyaluronic acid (Lee et al., 2007b) have been reported to show targeted silencing effects in cancer cells.

Despite the intensive investigation on developing efficient carriers for siRNA delivery, it has been difficult to overcome delivery hurdles due to the high toxicity of the cationic materials. Recently, some groups have reported studies on engineering the siRNA molecules for their enhanced delivery using less toxic but also less efficient carriers (Bolcato-Bellemin et al., 2007; Lee et al., 2010; Mok et al., 2010). A study by Mok et al. has reported the synthesis of reducible multimeric siRNAs, which are linearly cross-linked multimers of siRNA molecules linked by cleavable disulfide bonds (Mok et al., 2010). The multimeric siRNAs showed greatly enhanced gene silencing efficiencies compared to monomeric siRNAs when using the less toxic linear polyethylenimine as the carrier, due to the higher charge density and chain flexibility of the mulimerized siRNA conjugates. A more recent study has demonstrated that even dimeric conjugates of siRNA, just two siRNA molecules linked together, show gene silencing efficiencies comparable to the multimeric siRNAs (Chung et al., 2011). These studies suggest a novel approach in the development of strategies for siRNA therapeutics, also giving rise to new questions on the mechanisms of their delivery and function in the body.

Development of theragnostic nanomaterials

For cancer therapy, it is of great importance to detect the target site of tumor, as well as to monitor the bio-distribution and functions of the administered therapeutic agent *in vivo*. To achieve this, multi-functional nanomaterials that can simultaneously visualize and cure diseases have been intensively studied during the past several years (McCarthy and Weissleder, 2008). The theragnostic nanoparticulate systems are usually formed by using a contrast agent material (e.g. iron oxide nanoparticles) as the carrier for a therapeutic agent (e.g. small molecules, siRNA drugs, etc.). These systems can be designed to localize at the target disease site, which would allow the early detection of tumors, as well as trafficking them while they exert their therapeutic functions (Fig. 4). Especially, magnetic nanoparticles composed of iron oxide (Lee et al., 2008c), manganese oxide (Bae et al., 2011), and other doped composite nanomaterials (Lee et al., 2009b) have been widely investigated for cancer imaging due to their excellent contrast effects by magnetic resonance imaging (MRI) and therefore have been extensively studied as carriers for delivery of anti-cancer drugs.

Iron oxide nanoparticles have drawn attention for carrier-based drug delivery since they can be synthesized with high monodispersity, are biocompatible, and superior in imaging contrast (Park et al., 2004; 2005). Still, iron oxide nanoparticles have limitations in their instability and susceptibility to aggregation in aqueous media, and must be stabilized by modifying their surfaces using hydrophilic coating material or forming shell layers (Gupta and Gupta, 2005). The diverse studies on chemical modification of iron oxide nanoparticles have led to wider attempts to use these materials for drug delivery. A previous study has reported the development of multi-functional polymeric nanoparticles embedded with iron oxide nanoparticles and the anti-cancer drug, doxorubicin (Kim et al., 2008b). The polymeric matrix provided a reservoir for carrying the hydrophobic drug molecules which were efficiently delivered into cancer cells, while also showing high MRI contrast. Delivery of siRNA has also been attempted by adsorbing or conjugating the RNA onto the surface of coated iron oxide nanoparticles. A targeted nanosystem for siRNA delivery and MR imaging was developed by coating manganese-doped iron oxide nanoparticles with bovine serum albumin followed by chemical modification with RGD peptide and siRNA (Lee et al., 2009b). The multifunctionalized nanosystem showed high delivery efficiency into cancer cells in a target-specific manner, which resulted in exhibiting excellent MR imaging contrast and high gene silencing efficiencies.

Other than MRI, optical imaging techniques have also been introduced to nanoparticulate drug delivery systems. Especially, quantum dots have been widely used in nanoparticulate drug delivery systems to study the trafficking of the particles *in vitro* and *in vivo*, due to their sharp fluorescence and high photostability (Gao et al., 2004). Quantum dots that can deliver siRNA were formed by using a new class of cationic polymers with tertiary amine groups, which showed higher gene silencing efficiencies with low cytotoxicity compared to other conventional polymers such as PEI and Lipofectamine (Yezhelyev et al., 2008). A PEGylated quantum dot was used as a scaffold to conjugate siRNA and tumor homing peptide, which showed target-specific uptake into cells as well as efficient knock-down of a model gene (Derfus et al., 2007).

CONCLUSIONS

A variety of nanoscale drug delivery systems such as polymeric micelles and liposomes have been the subject of intensive research due to their unique characteristics for anticancer therapy. These nanomaterials exhibit greater cellular uptake, prolonged circulation after surface modification, and more efficient access to targeted tumor site, as compared with micron-sized particles. Moreover, they can carry a large number of therapeutic drugs owing to their high surface-area-to-volume ratio, penetrate into the leaky tumor vasculatures, and subsequently deliver the drug payloads to tumor tissues via the EPR effect. In addition, diverse nanoparticulate formulations based on polyelectolyte complex micelles and inorganic nanoparticles have been extensively explored to efficiently deliver siRNA to the target site for its desired functions. Another focus of recent research is the development of theragnostic nanosystems which can simultaneously visualize and treat diseases. In the near future, great advances in biomedical sciences and nanotechnology will expand the entities of artificially engineered nanomaterials for cancer therapy and imaging applications.

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