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Implementation of Nanotechnology as Tool for Preventing Cancer

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Abstract: Despite improvements in our understanding of pancreatic cancer and the emerging concept of personalized medicine for the treatment of this disease, it is still the fourth most common cause of cancer death in the western world. It is established that pancreatic cancer is a highly heterogeneous disease with a complex tumor microenvironment. Indeed the extensive stoma surrounding the cancer cells has been shown to be important in promoting tumor growth and metastases, as well as sequestering chemotherapeutic agents consequently decreasing delivery to the tumor cells. Nanotechnology has come to the forefront in the areas of medical diagnostics, imaging, and therapeutic drug delivery. This review will focus on the potential applications of nanotechnology for diagnosis, imaging, and delivery of therapeutic agents for the treatment of pancreatic cancer. The design and synthesis of nano-particles which can encapsulate and deliver a diverse range of therapeutic compounds—ranging from chemotherapy agents to DNA/RNA has received significant attention in cancer research. Nano-particles in the form of liposome's and/or polymer-derived nano-materials have been widely used in a number of pre-clinical cancer models. Importantly, these nano-particles have shown great potential as highly efficient delivery vehicles for chemotherapy drugs or RNA interference (RNAi) inhibitors and are currently being evaluated in human clinical trial. A select number of examples for the use of nano-particles to deliver chemotherapy agents or RNAi inhibitors are described in the following sections.

Keywords: Nanotechnology, Cytotoxicity, ATP-Binding Cassette, Tumor, Cancer, Inhibitors, Cells.

INTRODUCTION

Use of nanotechnology in medical science is a rapidly developing area. New opportunities of diagnosis, imaging and therapy have developed due to recent rapid advancement by nanotechnology. The most common areas to be affected are diagnostic, imaging and targeted drug delivery in gastroenterology, oncology, cardiovascular medicine, obstetrics and gynecology. Mass screening with inexpensive imaging might be possible in the near future with the help of nanotechnology. This review paper provides an overview of causes of cancer and the application of nanotechnology in cancer prevention, detection and treatment. Nanotechnology is a multidisciplinary field. It covers a vast array of devices that are derived from the areas of engineering, biology, physics and chemistry. These devices include vectors for the targeted delivery of anticancer drugs, imaging contrast agents and arrays for the early detection of tumors. These, and other nano-devices, may support the future diagnosis and treatment of common conditions such as cancer. Nanotechnology was first proposed in 1867 by James Clarke Maxwell, a Scottish physicist who developed electromagnetic theory and statistical physics. He suggested the concept of “Maxwell’s Demon”, an entity able to manipulate individual molecules. Nanotechnology was predicted by the Nobel Prize winner physicist Richard Feynman, in his talk “There is plenty of room at the bottom”, given at Caltech in 1959, he spoke of building factories that maneuvered things atom by atom. Later in 1974, this concept was defined by Professor Norio Taniguchi of Tokyo University of Science as the separation, consolidation, and deformation of materials by one atom or one molecule. The idea was

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then popularized in the 1980's by Dr. K. Eric Drexler, who suggested that it would be possible to build computers and robots far smaller than a single cell. Nanotechnology refers to structures that have been engineered at an atomic scale. Nanotechnology typically refers to structures up to 200 nanometers in size. As a system's size diminishes to nano-scale it leaves a Newtonian world and enters a 'quantum realm'. In this realm, particle behavior and processes are governed by quantum and statistical mechanics and atoms are manipulated by covalent, electromagnetic and Van der Walls forces. Structures made up of a monolayer (a sheet that is one atom thick), have properties that differ markedly from conventional experience. Opaque and stable metals, such as gold, are transformed into mono-layers that are transparent, inflammable catalysts. Nanotechnology is being used not only in the development of such catalysts, but additionally in semi-conductor technologies, such as memory storage, construction and household products such as sunglasses. Relating to cancer, nanotechnology is being applied in the following two broad areas, the development of nano-vectors – nano-particles which can be loaded with drugs or imaging agents and then targeted to tumor cells and nano-sensors - devices for the detection of cancer cells. Cancer is a genetic disease. In normal condition, cell division is controlled by the apoptosis complex. The apoptosis complex is activated by tumor suppressor protein P ^{53} and the tumor necrosis factor. When both mechanisms malfunction, cells undergo uncontrolled cell division and grow to malignant tumor. Capillaries grow abnormally within the tumor. The malignant tumor obtains nutrients from the surrounding healthy tissue. When the tumor is enlarged, some tumor cells enter the blood stream and eventually invade other parts of the body and form other tumors. This phenomenon is known as metastasis and is a fatal condition. Nanotechnology is an upcoming technology that may change current cancer diagnosis and treatment methods. Nanotechnology covers a broad range of topics; therefore, it is called a multidisciplinary field, which includes biology, physics, chemistry and engineering. The main idea in nanotechnology is fabrication and control of material at the nano-scale. Nanotechnology has provided a way for rearranging and restructuring matter on an atomic scale and hence that we can understand the root of any problem. Nanoparticles are microscopic particles with size in nanometers (1-100 nm). Nanoparticles can be classified depending on the major building material present. Depending on the building material, nanoparticles are of two types, organic and inorganic nanoparticles. Liposomes, dendrimers and carbon nanoparticles are good examples of organic nanoparticles. Quantum dots, gold (Au) containing nanoparticles (raman probes), SPIO nanoparticles, silver (Ag) oxide nanoparticles are examples of inorganic nanoparticles. Nanovectors and defined surface patterning are two major subfield of nanotechnology. Nanovectors are very important for the administration of targeted therapeutic and imaging agents. Liposomes are a good example of nanovectors. Nanoscale resolution is possible with electron beam lithography, ion beam lithography, layer-by-layer (LbL) assembly etc. Nanocantilevers and nanowires are good examples of multiplexing nanotechnology. Due to the unique properties of deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and proteins all are used as building blocks for bottom up nanofabrication. The mortor pRNA of bacteriophage phi29 forms dimers, trimers and hexamers. This phi29 pRNA can be altered to form a distinct shape and structure, which can be used as RNA array.

Table 1: Potential advantages of nanotechnology for the diagnosis and treatment of pancreatic cancer

Advantages of nanotechnology in diagnostics and imaging for pancreatic cancer	**Advantages of nanotechnology for therapy in pancreatic cancer**
Increased sensitivity and specificity compared to conventional assays using only small amounts of patient sample.	Increased drug delivery to tumor cells.
Detection of early cancer biomarkers in blood samples (RNA/DNA, exosomes, proteins).	Increased tumor specificity via the use of tumor cell targeting moieties.
Monitor patient treatment response via biomarker detection and/or imaging.	Potential to decrease off-target systemic drug toxicity.
Potential to non-invasively differentiate between tumor and stromal elements in pancreatic cancer.	Potential to deliver therapeutics to target and silence non-druggable genes using RNAi inhibitors.
Increased sensitivity to detect small local and distant metastases.	Provide increased solubility, stability and circulation half-life for current chemotherapeutic drugs.

METHODOLOGY: Obstacles to Cancer Treatment and the Potential of Nanotechnology

Cancer remains one of the main causes of death in humans and thus great efforts have been undertaken to develop cancer treatments. Cancer cells are notorious in their resistance to chemotherapy

in the clinic. In fact, an enormous body of research strongly suggests that drug-resistant cancer cells that remain alive after chemotherapy are responsible for the reappearance of tumors and the poor prognosis for patients. The occurrence of drug resistance leads to the failure of tumor treatment. This is a difficult obstacle to overcome, as tumor resistance mechanisms have various origins (Fig. 3.1). It is known that several members of the ATP-binding cassette (ABC) transporter family play an important role in cancer cell with resistance to different drugs. Studies have demonstrated that ABCB1, ABCC1, ABCG2, and other members of the ABC family are expressed in different types of human cancers, and their expression is related to the outcome of chemotherapy: the higher their expression in a tumor, the more resistant cancer cells are to chemotherapy. For example, a poor survival rate characterizes gliomas and tumor-derived endothelial cells that express ABCB1 and ABCC1 subfamily members. In the tumor tissue, tumor resistance can be connected to the physiology of the tumor tissue, including a poor vasculature and unsuitable physicochemical conditions.

To overcome drug resistance, many attempts have been made using strategies that consider the different chemotherapeutic mechanisms either at the cellular level or at the tissue level. In the clinic, multidrug resistance (MDR) occurs in over 50% of patients whose cancer relapses, accounting in large part for the high mortality associated with cancer. Tumor resistance to chemotherapy in the clinic can be due to the inefficient distribution of drug relative to its targeted tumor tissue. MDR may become evidence either as a lack of tumor size reduction or as a clinical relapse after an initial positive response to antitumor treatment.

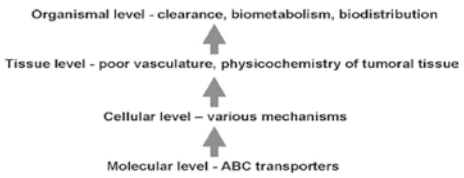

Figure 3.1: Mechanisms of clinical multidrug resistance during anticancer therapy

A number of mechanisms might be responsible for pleiotropic drug resistance in the clinic. Cellular resistance to chemotherapy is associated with overexpression of ABC membrane transporters such as Pgp, Mrp or BRCP that are responsible for “pumping” drugs out of cancer cell. Cytoskeletal disruption and other alterations prevent the correct localization of membrane proteins, and disrupted cell signaling in cancer cells may lead to drug resistance. Abnormal vasculature reduces the efficient biodistribution of anticancer drugs, resulting in less drug accumulation in the tumor. In clinical practice, drug resistance constitutes the failure of a patient to achieve a complete or partial response to therapy. In the laboratory, however, drug resistance is a cellular phenomenon and reflects the inability to demonstrate cytotoxicity at physiologically achievable drug concentrations in cancer cells. Drug resistance may be considered to be either intrinsic or acquired. Frequently, resistance is intrinsic to the cancer at the beginning, but as therapy becomes more and more effective, acquired resistance also becomes common. Intrinsic resistance occurs when tumor cells are capable of escaping exposure or repairing damage induced by the cytotoxic effects of chemotherapy at initial exposure. Finally, acquired resistance dominates when resistant cells survive from a population that was initially sensitive to chemotherapy. Both intrinsic and acquired resistance may operate along several different pathways, including decreased drug accumulation, decreased drug activation, increased repair of drug-induced damage, altered drug targets, altered gene expression and drug barriers. The development of resistance to chemotherapy is frequently associated with broad cross resistance even to structurally dissimilar drugs, suggesting the existence of more than one potential mechanism of resistance. Multiple changes often appear simultaneously in highly resistant tumor cell lines. This observation has led to the widely accepted hypothesis that tumor resistance to chemotherapy is usually multifactorial. Nanotechnology has the potential to overcome current obstacles to chemotherapy, because of the unique properties of nanoparticles (1–100 nm). For example, solid tumors have unique features, such as leaky tumor blood vessels and defective lymphatic drainage, that promote the delivery and retention of macromolecules or nanoscale particles, a phenomenon recognized as the enhanced permeability and retention (EPR) effect. Nanoparticles can be constructed at a certain size for effective biodistribution and accumulation in the tumor. Nanoparticles are characterized by self-assembly, stability, specificity, drug encapsulation and biocompatibility as a result of their material composition. Many researchers now make investigation to find out how to employ nanotechnology to overcome tumor multidrug resistance *in vivo* and *in vitro*.

Nanotechnology Allows Specific Targeting of Tumor

A major problem limiting the success of many anticancer agents lies in their inability to target tumor cells and tissues selectively. Therefore, almost all anticancer agents result in severe side effects to normal

tissues and organs. In chemotherapy, pharmacologically active concentrations of an anticancer drug in the tumor tissue are often reached at the expense of massive contamination of the rest of the body. This poor specificity creates a toxicological problem that represents a serious obstacle to effective antitumor therapy. Recently, progress has been made on the design of nanoparticles with surface properties that allow better accumulation in tumor tissue after systemic administration. To improve the specificity of nanoparticles, a molecular recognition moiety is connected to the surface of the nanoparticles to target cancer cells in tumor tissue after intravenous administration. For example, folic acid has been used to be conjugated to surface of nanoparticles. The rationale behind the choice of folic acid as a targeting moiety is that folic acid binding proteins are frequently overexpressed on the surface of human cancer cells. The folate-coated nanoparticles showed a tenfold higher apparent affinity for the folate binding protein than the free folate, as measured by surface plasma resonance. This increased apparent affinity was attributed to the fact that the particles represent a multivalent form of the folic acid ligand and can therefore display stronger interactions with the folate receptor. Thus, it could be expected that the folate-grafted nanoparticles would also strongly interact with the surface of malignant cells on which the folate binding protein can be overexpressed; such binding can eventually promote endocytosis of the nanoparticles mediated by folate binding protein. Indeed, only the cancer cells overexpressing the folate binding protein showed intensive uptake of the folate-decorated nanoparticles. The cancer cells that did not express the folate binding protein on the cell surface did not show any uptake of those nanoparticles. In addition, none of the cells was able to internalize PEG-coated nanoparticles without folate coating. The development of various nanoparticles with different ligands now offers a choice for targeted tumors with drug resistance. The suspension of nanoparticles is very stable, as evaluated by size measurements, and can be lyophilized. The surface properties, including the zeta potential, complement activation and protein adsorption pattern, are defined by the nature of the materials used to synthesize nanoparticles. Indeed, the biological activity of heparin grafted on the surface of nanoparticles was preserved at a level of 70% when compared to the activity measured for a heparin solution. The variety of biomolecules that can be conjugated to nanoparticles offers many possibilities for the design of targeted nanoparticles using a biomimetic approach. Chemotherapies should ensure a specific toxic effect against the targeted tumor cells, even if the increased complexity of the outer surface obstructs their diffusion into tumor. Utilizing a ligand that binds specifically to its receptor on a malignant cell may help to reduce the dose-limiting cytotoxicity of the drug and also enable the drug to bypass the drug resistance mechanism especially caused by P-glycoprotein (Pgp) overexpression, via internalization through receptor-mediated endocytosis. This strategy not only targets the malignant cells directly, but also aims at destroying nonmalignant tumor components that are crucial for tumor survival and development. Heparin-paclitaxel-Folic Acid with its highly specific tumor uptake and potent antitumor properties fits the profile of this strategic requirement very well. More recently, liposomes have been modified by conjugating them with monoclonal antibodies directed against tumor antigens. Furthermore, copolymer nanoparticles can form a shell with a hydrophobic inner part that contains the drug. They have a kinetic behavior similar to that of liposomes. These vectors may carry drugs, radioisotopes and/or labeling agents, and are directed against the specific surface structure of tumor cells, hence increasing the specific distribution and accumulation of drugs within tumors.

Nanotechnology Can Overcome Drug Resistance Due to Different Mechanisms

Drug resistance is known to develop through a variety of molecular mechanisms within the tumor (Fig. 3.1), and various approaches overcoming tumor resistance to chemotherapy are based on various pathways. For example, the enzyme glucosylceramide synthase (GCS), responsible for bioactivation of the proapoptotic mediator ceramide to a nonfunctional moiety glucosylceramide, is found to be overexpressed in many multidrug-resistant tumor types and has been implicated in cell survival in the presence of chemotherapy. In an attempt to circumvent the mechanisms that cancer cells use to avoid cell death following chemotherapy, a polymeric nanoparticle was created to deliver ceramide, which triggers resistant cells to apoptosis under paclitaxel treatment. Treatment with the multifunctional nanoparticle produced 100% mortality among cultured cells. To overcome MDR in a human ovarian cancer cell line, modified poly(epsiloncaprolactone) (PEO-PCL) nanoparticles were used to encapsulate and deliver therapeutic agents for enhanced efficacy. With nanoparticle drug delivery, the resistant cells can be sensitized to paclitaxel near the IC50 concentration of sensitive cells. Chemotherapy enhanced via nanoparticle delivery has a promising potential as a strategy to overcome MDR. Tumor cells can develop simultaneous resistance to multiple anticancer drugs. An alternate strategy suggested for overcoming MDR is association of the drug with nanoparticles. The rationale behind this strategy is to increase the intracellular concentration of the drug and other agents using endocytosis. Doxorubicin, an anticancer drug widely used in cancer therapy and a known substrate of Pgp, was encapsulated in various types of

nanoparticles. The sensitivity of resistant cells to the doxorubicin-loaded nanoparticles was then evaluated by measuring the cytotoxic effect produced by increasing the concentration of the doxorubicin-loaded nanoparticles. Resistant cells treated with alginate or lactide-co-glycolide modified nanoparticles showed the same sensitivity to the treatment as the free drug. In contrast, resistant cells treated with doxorubicin-loaded poly(alkyl cyanoacrylate) (PACA) nanoparticles showed a much higher sensitivity to the drug, relative to the free drug. The sensitivity of the resistant cells even reached the level of sensitivity of the parent sensitive cells, suggesting that the PACA nanoparticles can totally overcome the tumor resistance to doxorubicin. To explain the ability of nanoparticles to overcome doxorubicin resistance, researchers have proposed a mechanism based on the adhesion of the nanoparticles to the surface of resistant cancer cells. Adhesion is followed by the simultaneous release of the drug and nanoparticle degradation products that pass through the cell membrane without being recognized by Pgp. To circumvent MDR, some proposed the use of competitive inhibitors such as verapamil. However, the clinical use of verapamil to overcome MDR is limited due to the serious adverse effects of this compound. More recent studies that have been designed to further improve the efficacy of nanoparticles in overcoming MDR have been based on limiting the activity of Pgp. This strategy is also an interesting alternative to promote the efficacy of doxorubicin-loaded nanoparticles. Soma et al. suggested co-encapsulating doxorubicin and cyclosporin A within the nanoparticles. Cyclosporin A is a chemosensitizing compound that can bind to Pgp and inhibit the pump efflux activity. Doxorubicin was incorporated within the core of the nanoparticles while cyclosporin A was located at the nanoparticle surface. Using different formulations of the drug-loaded nanoparticles, it was shown that the association of both doxorubicin and cyclosporin A within a single nanoparticle led to the most effective growth rate inhibition of the resistant cells. The association of cyclosporin A with doxorubicin nanospheres would also ensure that cyclosporin A reaches the same sites with the anticancer drug and also reduces its toxic side-effects. Other strategies proposed to regulate the expression of the Pgp have involved using siRNA. However, the results obtained were disappointing because of the long half-life of Pgp, making its down-regulation difficult.

Engineered Nanoparticles Facilitates Targeting of Tumors

Nanoparticles have the potential to enhance the protection of drugs against biotransformation and rapid clearance *in vivo*. In order to do so, they must have long-circulating properties to reach the tumor tissue. In addition, they should have the proper biodistribution to target the tumor. With these objectives, studies have focused on customization of the surface properties of nanoparticles. Researchers have sought to modify the nanoparticle biodistribution to target tumors using poly(ethylene glycol) (PEG) as a coating material at the nanoparticle surface in order to reduce protein adsorption and complement activation. PEG-coated nanoparticles were prepared from a poly(PEG cyanoacrylatecohexadecyl cyanoacrylate) copolymer. These nanoparticles circulated longer in the blood stream, while their uptake by the liver was reduced. They were found to accumulate in the brain to a larger extent than other formulations, including the non-PEG-coated nanoparticles. The concentration of PEG-coated nanoparticles in the central nervous system was shown to be greatly increased, especially in the white matter when compared to conventional nanoparticles. Recently, these nanoparticles were shown to accumulate specifically in a glioma implanted into a rat brain. The accumulation was found to occur mainly in the tumoral tissue, while the amount of nanoparticles found in the adjacent healthy tissue and in the control hemisphere was much lower. The comparable distribution in tumor and normal tissue was attributed to the difference in the microvascular permeability between healthy and tumor tissue, combined with an increased circulation time in the blood stream. Maeda et al. found that Evans blue dye, which binds with plasma albumin, concentrated selectively in tumor tissues following intravenous (i.v.) injection. The same behavior was also noticed with radiolabeled plasma proteins, including transferrin (90 kDa) and IgG (160 kDa), whereas smaller proteins such as neocarzinostatin (12 kDa) did not accumulate in tumors. The tumor accumulation reaches up to several fold higher than that of the plasma due to lack of efficient lymphatic drainage in the solid tumor; this provides an ideal application for EPR-based selective anticancer drug delivery and distribution in a tumor. Tumor blood vessels are thought to have relatively large pore structures and poorly aligned defective endothelial cells lacking a smooth muscle layer. Extensive production of vascular permeability enhancing factors, such as nitric oxide (NO), lead to highly abnormal transport dynamics across tumor capillaries, especially for nanosized macromolecular drugs. Thus, it becomes possible for anticancer nanomedicines of certain sizes to cross selectively into tumor tissues. Furthermore, tumor tissues usually lack effective lymphatic drainage, which leads to prolonged retention of nanoparticles. Due to their size, nanoscale particles containing anticancer drugs administered intravenously (i.v.) can escape renal clearance. Often they cannot penetrate the tight endothelial junctions of normal blood vessels, but can extravasate in tumor

vasculature and become trapped in the tumor vicinity. Establishment of this principle hastened the development of various multifunctional nanoparticles for targeted cancer chemotherapy. Indeed, this highly selective local distribution of nanoparticles in tumor tissues has proven superior in therapeutic effect with minimal side effects in both preclinical and clinical settings. Gabizon et al. found that 100 nm nanoparticles can passively enter tumor tissues, thereby increasing selectivity of anticancer drug delivery at the tumor site, while markedly reducing drug accumulation and toxicity in many susceptible healthy tissues. If the level of drug resistance is comparable to the drug levels in tumor, MDR may be overcome by increasing delivery of anticancer drugs based only on mass action. Biocompatible and sterically stabilized micelles (SSMs) have been used as nanocarriers for chemotherapeutic agents. Drug solubilization in SSMs is reproducible and is attributed to the avoidance of drug aggregate formation. Furthermore, SSMs composed of polyethylene glycol (PEGylated) phospholipids are attractive nanocarriers for drug delivery because they are sufficiently small (14 nm) to cross through the leaky microvasculature of tumors and penetrate tissues for passive targeting of solid cancers *in vivo*, resulting in high drug concentration in tumors and reduced drug toxicity to the normal tissues.

Nanoparticle Properties Improve Drug Accumulation in Tumors

During the past few years, several strategies have been investigated to improve the clinical effectiveness of chemotherapy; in particular, attention has been focused on drugs and their pharmaceutical formulations. A promising strategy is the use of carriers to transport drugs that are already employed in the clinic such as platinum complexes. Nanoparticles and liposomes represent two major formulations that are in active clinical evaluation. The EPR effect allows significant increase in drug concentration; therefore, the cytotoxicity against tumor cells is increased, while normal tissues are spared from the drug-induced damage. However, it is evident that the incomplete and immature vasculature within the tumors plays a fundamental role in drug resistance. The immature vasculature leads to reduced oxygenation and nourishment of cancer cells, and cancer cells adapt to grow in these critical conditions. The adaptation leads to changes in gene expression and metabolic pathways, which contributes to diminishing pH values in the tumor until an acidic pH is achieved and maintained. In these conditions, drug resistance phenomena may begin to occur because many drugs become ionized due to their pKa values within the range from 5.8 to 8.5. Weak basic drugs, such as anthracyclines and vinca alkaloids, diffuse poorly in an acidic extracellular milieu because their ionized status obstructs their passage through cell membranes. Low pH may cause tumor resistance to mitoxantrone, a weak basic drug. Conversely, weak acid agents, such as chlorambucil and 5-fluorouracil, have an advantage in terms of distribution within the tumor and cytoplasmic sequestration because of the neutral-to-alkaline pHi. Polymer micelles as powerful chemotherapeutic nanoparticles afford several advantages for targeted drug delivery in cancer, including increased drug solubility, prolonged circulation half-life, selective accumulation at tumor sites, and a decrease in toxicity. However, the technology still lacks tumor specificity and controlled release of the entrapped agents. Therefore, the focus has gradually shifted from passive targeting micelles to active targeting and responsive systems that carry additional mechanisms for site-specific release. pH-sensitive formulations are examples of how the versatility of micelles can lead to a fusion of chemical customization with biological insight to achieve active drug delivery.

In addition, hyperthermia may also increase drug accumulation within a tumor and has been evaluated in association with liposomes. Hypoxia may also exert a significant influence on drug sensitivity through the modulation of mRNA levels of several genes. For example, the chronic influence of hypoxia may lead to etoposide and vincristine resistance by modifying gene expression of HIF-1α. Furthermore, hypoxic conditions increase the heme content and induce the expression of ABCG2 protein in stem cells. This induction allows cellular survival by removing heme from the cytoplasm and thus diminishing the formation of reactive oxygen species (ROS). Therefore, the hypoxia-induced expression of ABCG2 seems to give a double advantage to cancer cells, allowing survival in critical conditions and making the tumor resistant to drugs. Modification of extracellular (pHe) and intracellular (pHi) could help to reverse drug resistance in tumors. Recent studies have demonstrated that some drugs exert their cytotoxic effects by altering the regulation of pHi through production of H_2O_2 in the mitochondria. Furthermore, an acidic pHi increases tumor sensitivity toward several drugs. Recent data support the use of proton-pump inhibitors (PPIs) to increase pHe and the pH of lysosomal organelles. Pretreatment with PPIs may reverse the MDR tumor phenotype, likely through the inhibition of drug excretion by ABC family members (i.e., ABCB1 or ABCG2). In some cases, it has been hypothesized that PPIs could induce drug accumulation within vesicle-like structures which cannot be excreted. Bioreductive drugs represent a logical consequence in the drug development process based on the knowledge of biologic characteristics of tumors. In contrast with other bioreductive drugs, tirapazamine is active at intermediate oxygen concentrations, which acts synergistically with several antineoplastic agents such as cisplatin. Block-copolymer micelles are

spherical supramolecular assemblies of amphiphilic copolymers that have core–shell architecture. The core is a loading space that can accommodate hydrophobic drugs, and the shell is a hydrophilic brush-like corona that makes the micelle water soluble. Block-copolymer micelles allow delivery of poorly soluble contents and avoid the pHe and pHi limitation. The micelles localize in several cytoplasmic organelles, including the mitochondria, but not the nucleus. Administering immunomicelles loaded with the anticancer drug taxol to mice with lung carcinoma resulted in increased accumulation of taxol in the tumor. Furthermore, nanoparticle shape may be important in designing better nanotechnology-based drug delivery vehicles. Filomicelles are about ten times longer than their spherical counterparts and are more persistent than any known synthetic nanoparticle. Preliminary results further demonstrate that filomicelles can effectively deliver the anticancer drug paclitaxel and shrink humanderived tumors in mice. Although these findings show that long-circulating vehicles need not be nanospheres, they lend insight into possible shape effects on nanoparticle function.

Nanoparticles Used for Tumor Treatment

Because cells will typically internalize nanomaterials below 100 nm, nanostructures have the ability to enter the cells due to their nanoscale size. Some of the leading nanostructures being used for this purpose include fullerenes, dendrimers, and nanoshells (Table 3.1). Fullerenes (or Buckyballs) are natural hollow spheres, 1 nm in diameter, made with carbon atoms. Fullerenes create a unique drug delivery platform that allows active pharmacophores to be conjugated to their surface in three-dimensional orientations for precise control in matching fullerene compounds to biological targets, in entrapping atoms within the fullerene cage, and for attaching fullerene derivatives to targeting agents. One of these fullerenes investigated by our group is $[Gd@\ C_{82}(OH)_{22}]_n$, which is a water-soluble hydroxyl modified metal-fullerene.

This nanoparticle has a strong capacity to enhance immunity and protect the normal tissues from tumor invasion, with almost no toxicity *in vivo* and/or *in vitro*. In comparison with conventional antitumor chemicals such as cisplatin and cyclophosphamide, this nanoparticle is highly efficient at suppressing tumor growth. Its action is not due to toxic effects on tumor cells because it does not affect tumor cell proliferation directly under the administrated concentration. The distribution in the tissues is mainly in bones (about 1% of administration), then the pancreas, kidney and spleen, in that order. About 50% of $[Gd@C_{82}(OH)_{22}]_n$ are excreted in the urine and 35% in the feces, which suggests that this nanoparticle reaches tissues and organs through blood circulation and does not remain in the blood after 24 h of administration. This could be improved by appropriate modification of this nanoparticle.

Table 3.1: Nanomaterials potential for nanomedical application

Subjects	**Properties**	**Nanomaterials**
Nanocrystals	Materials with nanocrystalline structure are different in their atomic structure, crystallographic orientation, or chemical composition	Ceramic, metal (quantum dots, nanogold, nanosilver, etc.) and metal oxide nanoparticles (CuO, ZnO, TiO_2, SiO_2, etc.)
Carbon nanotubes/fullerenes	Carbon-based nanomaterials are composed mostly of carbon in the form of a hollow spheres, ellipsoids, or tubes etc. Fullerenes are characterized with an elongated sphere of carbon atoms formed by interconnecting six-member rings and 12 isolated five-member rings forming hexagonal and pentagonal faces	Hollow cylinders of carbon atoms such as: Carbon nanotubes (CNTs) Fullerences including C60, C70, C80, Gd@C82, C84, etc.
Organic nanoparticles	Nanomaterials with three components: a central core, an interior dendritic structure (the branches), and an exterior surface (the end groups)	Polymers built from branched units with numerous chain ends on the surface such as various dendrimers
Inorganic–organic hybrid nanoparticles	Hybrid nanomaterials consist of one material as matrix filled with another material	Nanoparticles or nanofibers with at least two different materials such as polyhedral silicon-containing organic polymers

Another nanomaterial used for cancer treatment is the dendrimer, which was used to treat tumor cells without triggering an immune response. This is due to the dendrimer's small size and branched structure. Dendrimers can be designed to release attached compounds in response to a specific molecule or chemical reaction. In addition, a layered sphere called nanoshell is being developed for cancer therapy. The nanoshell has a gold exterior layer which covers interior layers of silica and drugs. Nanoshells can be made to absorb light energy and then convert it to heat. As a result, when nanoshells accumulate next to a target area such as a tumor cell, they can release tumor-specific antibodies when infrared light is administered. Successful design of nanoparticles to treat tumors effectively requires assembly of the appropriate targeting ligands on nanocarriers and long-circulating nanosystems with appropriate surface modification and the capability to control particle stability, aggregation, receptor binding and subsequent biochemical cascades and signaling processes. The size of the particles must be large enough (30–100 nm) to avoid leakage into blood capillaries but not so large (>100 nm) that they become susceptible to macrophage-based clearance. Surface manipulation can control the particle aggregation at interstitial sites, optimizing nanoparticle retention at lymph nodes. Very small particles (1–20 nm) with long circulatory residence times could slowly penetrate the vasculature into the interstitial spaces, and be transported by lymphatic vessels to lymph nodes. This phenomenon is quite important when designing nanoparticles to allow differential leakage from the blood circulation system through the permeable endothelium in lymph nodes. To date, many different nanoparticles have been synthesized and developed for effective treatment of tumors (Table 3.1).

Recently, a multistage nanoparticle system has been employed. This multistage system consists of mesoporous material and nanoparticles as two major components. Modified mesoporous material is about to carry nanoparticles to their designated targeting site; it then degrades to release the nanoparticles into the targeted tissue. The released nanoparticles consequently merge into cells for efficient treatment.

Nanotechnology Can Improve the Bioavailability of Poorly Soluble Anticancer Drugs

Nanotechnology has been successfully utilized to create a new drug delivery system that can solve the problem of poor water solubility common to many promising and currently available anticancer drugs and thereby increase their effectiveness. The poorly soluble anticancer drugs require the addition of solvents in order for them to be easily absorbed into cancer cells. Unfortunately, these solvents not only dilute the potency of the drugs but increase toxicity as well. Silica-based nanoparticles are used to deliver hydrophobic anticancer drugs and other water-insoluble drugs to human cancer cells. The experimental results suggest that mesoporous silica nanoparticles might be used as a vehicle to overcome the insolubility problem of many anticancer drugs. Paclitaxel is widely used to treat multiple types of solid tumors. The commercially available paclitaxel formulation uses cremophor/ethanol (C/E) as solubilizers. Other formulations including nanoparticles have also been introduced. The nanoparticle and C/E formulations showed significant differences when compared to paclitaxel itself. Tissue specificity of the two formulations was different too. The nanoparticles showed longer retention and higher accumulation in organs and tissues. The most striking difference was an eightfold greater drug accumulation and sustained retention in the kidney. These data indicate that the nanoparticulate formulation of paclitaxel affects its clearance as well as distribution in tissues with preferential accumulation in the liver, spleen, small intestine, and kidney. As mentioned above, block-copolymer micelles with core– shell architecture provide a loading space that can accommodate hydrophobic drugs, and the shell is a hydrophilic brush-like corona that makes the micelle water soluble, thereby allowing delivery of the poorly soluble contents and accumulated in tumor. Other nanoparticles consisting of human serum albumin (HSA) and containing different antisense ODNs (ASOs) have also been used for drug delivery insoluble drugs. The preparation process was optimized regarding the amount of desolvating agent, stabilization conditions, as well as nanoparticle purification.

Wartlick et al. found that the glutaraldehyde cross-linking procedure of the particle matrix was a crucial parameter for biodegradability and drug release of the nanoparticles. The drug loading efficiency increased with longer chain length and employment of a phosphorothioate backbone. It indicated that there was no cytotoxic effect observed under nanoparticle concentrations up to 5,000 μg/ml in different tumor cells.

In this study, the entrapment of a fluorescent labeled oligonucleotide within the particle matrix was used to detect intracellular drug release of the carrier systems. It was revealed under confocal laser scanning microscopy that nanoparticles cross-linked with low amounts of glutaraldehyde could rapidly be degraded intracellularly and could lead to a significant accumulation of the ASO in cytosolic compartments of the tumor cells.

Resistance to Cisplatin: A Broadly Used Anticancer Drug

The platinum coordination complex known as Peyrone's chloride was firstly synthesized and described by M. Peyrone in 1845; these findings were published in 1965. In the 1960s, Barnett Rosenberg serendipitously discovered its chemotherapeutic cancer activity. In 1968, following further tests against various bacteria, cisplatin was administered intraperitoneally to mice at the nonlethal dose of 8 mg/kg, and was shown to cause marked tumor regression. The patient was first treated with confirmatory *in vivo* tests performed by clinical testing in 1971. Cisplatin was approved by the US Food and Drug Administration (FDA) for clinical application in 1978. Since the biological properties of cisplatin as a anticancer drug were accidentally discovered over 40 years ago, it has had a major impact on the chemotherapeutic treatment of various cancers and is still widely used today. Cisplatin is one of the most widely used and most effective cytotoxic agents, and is broadly employed in the treatment of epithelial malignancies such as lung, head and neck, ovarian, bladder and testicular cancer. The action mechanism of cisplatin involves covalent binding to purine DNA bases, which primarily leads to cellular apoptosis. However, its continued clinical use is impeded by its severe adverse reactions including renal toxicity from renal tubular damage, gastrointestinal toxicity, peripheral neuropathy, asthenia, and ototoxicity.

The major limitation in the clinical applications of cisplatin is the development of cisplatin resistance by tumors. This arises either by clonal expansion of tumor cells in the heterogeneous tumor cell population with inherent resistance to cisplatin (with mutations in specific genes that confer resistance), or by acquired resistance by some cells in the tumor during treatment and their clonal expansion after killing of the sensitive cells by the drug. Tumor proliferation could be mainly conferred by limited uptake of the cisplatin by drug-resistant cells. Much is currently understood about how tumors commonly exhibit resistance to cisplatin, either intrinsically or as acquired during the courses of therapy. Mechanisms explaining cisplatin resistance include the reduction in cisplatin accumulation inside cancer cells because of barriers across the cell membrane, the faster repair of cisplatin adducts, increased cytoplasmic detoxification and tolerance to DNA damage, the modulation of apoptotic pathways in various cells, the mislocalization of functional membrane protein and a higher concentration of glutathione and metallothioneins in some types of tumors. A number of experimental strategies to overcome cisplatin resistance are at the preclinical or clinical stages.

Increased Intracellular Cisplatin Accumulation to Reverse Tumor Resistance

Reduced cisplatin intracellular accumulation is the common result in different types of cisplatin-resistant cell lines. Cisplatin is highly polar and enters cells relatively slow in comparison to other classes of small-molecule cancer drugs. The uptake of cisplatin is influenced by factors such as sodium and potassium ion concentrations, pH, and the presence of reducing agents. The role of transporters or gated channels has been postulated in addition to passive diffusion. So far, copper transporter-1 (CTR1) is considered to have a substantial role in cisplatin influx. Loss of CTR1 was found to lead to a two- to threefold increase in drug resistance. In contrast to the mechanism of MDR, which is caused by the overexpression of ABC transporters, it is generally decreased uptake rather than increased efflux that predominates in cisplatin-resistant cells. The efflux proteins such as multidrug resistance protein-1 (MRP1, also known as ABCC1), MRP2 (also known as CMOAT or ABCC2) was reported to be partially associated with cisplatin resistance. The conjugation of cisplatin with glutathione was more readily exported from cells by the ATP-dependent glutathione S-conjugate export (GS-X) pump (that is, MRP1 or MRP2). Other studies also support a role for the glutathione metabolic pathway in acquired and inherited drug resistance to cisplatin. Maintenance of cisplatin levels in tumors for prolonged periods is expected to eradicate cisplatin sensitive cells without offering them a chance to develop resistance. Hurdles are the side effects of cisplatin and the toxicity from the cumulative dose. Several mechanisms can contribute to cisplatin resistance. A common observation, repeatedly reported over many years in many tumor cells with acquired resistance to cisplatin, is that of reduced platinum accumulation in comparison to the parental cells. The reduction in cisplatin accumulation inside cancer cells because of the cell membrane barrier is currently considered a major mechanism of acquired cisplatin resistance. The copper transporter CTR1 appears to control the accumulation of cisplatin in *Saccharomyces cerevisiae*. CTR1-deficient cells have reduced the uptake of cisplatin, and are 1.9-fold more resistant to the cytotoxic effect of cisplatin. However, until recently, the underlying complex molecular mechanism by which cisplatin enters cells still remained poorly defined. Drug delivery in cancer is important for optimizing the effect of drugs and reducing toxic side effects. Several nanobiotechnologies, mostly based on nanoparticles, have been used to facilitate drug delivery in cancer. The development of less toxic, nanoscale liposomal formulations of cisplatin has been hampered by the low water solubility and low lipophilicity of cisplatin, resulting in very low encapsulation efficiencies. Burger et al. reported a novel method to efficiently

encapsulate cisplatin in a lipid formulation by repeated freezing and thawing. The method is unique in that it generates nanocapsules, which are small aggregates of cisplatin covered by a single lipid bilayer. The nanoparticles have an unprecedented drug-to-lipid ratio and an *in vitro* cytotoxicity higher when compared to free cisplatin. It suggests that the nanoscale encapsulation may also be generalized to other drugs with low water solubility and lipophilicity. A polymer–metal complex formation between cisplatin and PEG-poly(glutamic acid) block copolymers were prepared before by Nishiyama et al., and their utility was also investigated as a tumor-targeted drug delivery system. Cisplatin-incorporated micelles with a 28 nm size exhibited a sustained drug release and the decay of the carrier itself in physiological saline. These nanoscale micelles showed a remarkably prolonged blood circulation and effectively accumulated in solid tumor sites. These data suggest that micelles with cisplatin could be a promising formulation for the targeted therapy of solid tumors. Micelles with a hydrophobic inner core and hydrophilic outer shell allow the chemical entrapment of cisplatin into the micelles; cisplatin is then released slowly into the target organism. As the extracellular pH of solid tumors has often been shown to be more acidic than normal tissues, this might also explain, in part, the increased tumor delivery of micelles with cisplatin. Overall, numerous mechanisms seem to be involved in tumor resistance to cisplatin studied *in vitro*. Studies have provided several rational approaches to circumventing clinical cisplatin resistance in patients. The strategy of using delivery vehicles to selectively transport more of a tumor-killing agent to tumors is attractive, and has now been clinically validated with the cytotoxics doxorubicin (liposomal doxorubicin) and paclitaxel (nanoparticle albumin-bound paclitaxel). To exploit the EPR effect of cisplatin in tumors, it has been linked to water-soluble, biocompatible nanomaterials. Trials are continuing with a reformulated cisplatin in an attempt to improve its antitumor activity.

Circumventing Cisplatin Resistance by Nanotechnology-Based Delivery

Chemotherapy patients can be classified as either platinum-sensitive or platinum-resistant, depending on whether they have relapsed or progressed within 26 weeks of completing first-line platinum based chemotherapy. Expression of the mitogen-activated protein kinase phosphatase-1 (MKP-1) was a prognostic marker for patients with invasive ovarian carcinomas. The MKP-1 mRNA levels were strongly inducible upon treatment of OVCAR-3 cells with cisplatin. MKP-1 expression is a clinically useful marker to estimate patient prognosis as well as response to cisplatin chemotherapy. Nanotechnology can be applied to encapsulate and protect drugs during transit *in vivo*. Drug encapsulation materials include liposomes and polymers (i.e., Polylactide (PLA) and Lactide-co-Glycolide (PLGA)). In addition to liposomes and polymers, other types of nanoparticles are also available for encapsulation. Materials such as silica and calcium phosphate (hydroxyapatite) have demonstrated superior properties at nanoscale rather than microscale, and can potentially be better suited for cisplatin delivery challenges. The materials form capsules around cisplatin and permit timed drug release to occur as the drug diffuses through the encapsulation material. Lipoplatin is a liposomal cisplatin formulation currently under clinical trials. The advantage of lipoplatin appears to arise from its 2- to 50-fold higher concentration in human tumors when compared to normal human tissues in biopsies, measured as total platinum with atomic absorption. The lipoplatin formulation can attain a higher concentration in tumors via its preferential extravasation through altered and compromised tumor vasculature. In order to achieve this property, liposomes that enclose chemotherapy drugs must have a diameter below 130 nm, long-circulation properties and the ability to escape immune surveillance.

The antitumor activity of cisplatin, encapsulated into transferrin-conjugated polyethylene glycol liposomes (Tf-PEG liposomes), was studied in nude mice with peritoneal dissemination of human gastric cancer cells. Small unilamellar Tf-PEG, PEG or DSPC/CH liposomes (bare liposomes) encapsulating cisplatin were prepared by reverse-phase evaporation followed by extrusion. The Tf-PEG liposomes were internalized into tumor cells by receptor-mediated endocytosis as shown by electron microscopy. Uptake of Tf-PEG liposomes into the liver and spleen was significantly lower than that of bare liposomes and had anti-tumor properties in nude mice xenografts that were better than free cisplatin. A novel bile acid–cisplatin complex, called as Bamet-R2, with liver vectoriality, was synthesized with the aim of overcoming cisplatin resistance. This complex had increased water solubility by encapsulation into liposomes and enhanced uptake by liver tumor cells. Bamet-R2 was effectively incorporated into liposomes with an increase in the concentration of the drug by more than 6 million fold compared with that in the initial free solution; this is 1,000-fold higher than the encapsulation obtained for cisplatin. A lipophilic cisplatin derivative, NDDP, formulated in conventional liposomes was shown not to be a cross-resistant with cisplatin in different *in vitro* and *in vivo* systems, and more active than cisplatin against tumor metastasis. NDDP was also formulated in liposomes composed of phosphatidylcholine, cholesterol and monosialoganglioside or PEG conjugated to phosphatidylethanolamine with prolonged circulation.

Nanoparticles Employed for Cisplatin Delivery in Preclinical and Clinical Stage

Local and sustained release of cisplatin near or inside a tumor may have distinct advantages over systemic administration of the drug. Cisplatin formulations in gel-type materials suitable for intratumoral injection have been tested in several laboratories. In general, these methods suffer from inefficient loading of the drug and other hurdles relating to its release mode and overall toxicity of the formulation. Malignant bone tumors are treated with surgical therapy and simultaneous systemic chemotherapy. In order to overcome the toxicity of this approach, the bone-cementing apatite (calcium phosphate) was used for a cisplatin formulation to develop an implant and maintain high concentrations of cisplatin at local sites in animals to counteract local structural weakness after tumor resection and treat residual malignant bone tumors. Approximately 33% of the total bound cisplatin was released after 4.25 days. This approach might be used for the slow and local release of cisplatin *in vivo*. PLGA-mPEG nanoparticles containing cisplatin were prepared by a double emulsion method and characterized with regard to their morphology, size, zeta potential and drug loading. Although intravenous administration of these cisplatin nanoparticles in mice resulted in prolonged cisplatin circulation in the blood, they suffered from loading efficiency for therapeutic applications. Degradable starch microspheres in an aqueous crystal suspension were used in clinical trials to achieve intensification of intraarterial chemotherapy of head and neck cancer with high-dose cisplatin. Nanoparticle-formulated cisplatin might further broaden its applicability to tumor types such as prostate cancer and small-cell lung cancer. Improved tumor delivery strategies and controlled release of cisplatin with specific modulators of cisplatin-resistance mechanisms might also provide future clinical benefits. Such strategies are sometimes unsuitable for clinical practice because of technical and biologic constraints, but in some cases they represent fruitful efforts to improve cancer chemotherapy. In particular, novel pharmaceutical formulations of cisplatin improve treatment efficacy and tolerability by increasing drug delivery within tumors or in close proximity.

Prospective Application of Nanotechnology to Reverse Tumor Resistance

Nanotechnology provides a wide range of new technologies for developing customized solutions that optimize the delivery of pharmaceutical agents. To be therapeutically effective, drugs need to be protected during their transit to the target action site *in vivo* while maintaining their biological and chemical properties. Some drugs are highly toxic and can cause serious side effects and have reduced therapeutic effect if they decompose during their delivery. Once the drug arrives at its destination, it needs to be released at an appropriate rate so that it can be effective. If the drug is released too rapidly it may not be completely absorbed, or it may cause gastrointestinal irritation and other side effects. The use of nanoparticle for drug delivery could positively impact the rate of absorption, distribution, metabolism, and excretion of the drugs in the body. In addition, nanoparticle delivery can allow the drug to reach its target in a more active form. There are severe restrictions on the nanomaterials and synthesis processes that can be used in drug delivery systems. The drug delivery material must be compatible and has to be easily bound with the drugs; in addition, the nanomaterial has to be easily degraded after use. It can be either metabolized or eliminated via normal excretory routes.

Nanotechnology can offer new drug delivery solutions by drug encapsulation. When materials are encapsulated in nanoparticles within the 1–100 nm size range, they have a larger surface area for the same volume, smaller pore size, improved solubility, and different structural properties. This can improve both the diffusion and degradation characteristics of the encapsulated nanomaterial. Nanotechnology involves the creation and use of materials and devices at the atomic and molecular level. Because clinical chemotherapy uses a variety of molecular materials and devices, and nanotechnology has the potential to provide many medical and pharmaceutical insights, such as how molecular materials self-assemble, self-regulate, and self-destroy. The scope of nanotechnology is enormous and it overlaps with the traditional medicine. Although only a subset of nanotechnology is applied to biological processes including medical and pharmaceutical usage, the potential for breakthrough is enormous and is being pursued on multiple fronts.

DISCUSSION

Conventional chemotherapeutic drugs are distributed non-specifically in the body where they affect both cancerous and healthy cells, resulting in dose-related side effects and inadequate drug concentrations reaching the tumor. Non-specific drug delivery leads to significant complications that represent a serious obstacle to effective anticancer therapy. In addition, the occurrence of resistance phenomena reduces the efficacy of cancer treatment. To overcome the lack of specificity of conventional chemotherapeutic drugs, several ligand-targeted therapeutic strategies, including immunotoxins, radioimmunotherapeutics, and drug immunoconjugates, are being developed. Although these conjugated

agents have shown promising efficacy compared with conventional chemotherapy drugs, limitations in their delivery efficiency still remain. Recent progress in cancer nanotechnology raises exciting opportunities for specific drug delivery. Nanoparticles, particularly in the size range from 10 nm to 100 nm, are emerging as a class of therapeutics for cancer treatment. Nanoparticles can be composed of several functional molecules simultaneously, such as small molecule drugs, peptides, proteins, and nucleic acids.

By using both passive and active targeting strategies, nanoparticles can increase the intracellular concentration of drugs in cancer cells while minimizing toxicity in normal cells; thereby enhancing anticancer effects and reducing systemic toxicity simultaneously, when compared with the therapeutic entities they contain. Furthermore, nanoparticles offer the potential to overcome drug resistance, since nanoparticles can bypass the P-glycoprotein efflux pump, one of the main drug resistance mechanisms, leading to greater intracellular accumulation. The purpose of this review article is to summarize the results of the use of therapeutic nanoparticles in the clinic and discuss the opportunities and challenges faced by therapeutic nanoparticles. Thus, the first part will emphasize the key properties of therapeutic nanoparticles and how these properties affect the efficiency and specificity of nanoparticles as a drug delivery system. Next, we will summarize current clinical uses of the first-generation therapeutic nanoparticles and the advances in new generation of therapeutic nanoparticles currently under preclinical and clinical investigation. Finally, we will discuss how nanoparticles will be developed to improve their therapeutic efficacy and function for future cancer treatment.

Challenges in Cancer Nanotechnology

In an ideal scenario, the onset of the transformational processes leading towards malignancy would be detected early. This could exist as routine screening by non-invasive means such as proteomic pattern analysis from blood samples, or the in vivo imaging of molecular profiles and evolving lesion contours. The biology of the host and the disease would be accurately determined, and dictate choices for targeting and barrier-avoiding strategies for an intervention plan. Transforming cellular populations would be eradicated or at least contained, without collateral effects on healthy tissues, in a routine that could be repeated many times. Treatment efficacy would be monitored in real time. Therapeutics would be supplanted by personalized prevention. If fully integrated with the established cancer research enterprise, nanotechnology might help this vision become reality. Some of the principal challenges along this path are as follows:

- Developing approaches for the in vivo detection and monitoring of cancer markers
- Improvement of the targeting efficacy of therapeutic or imaging agents to cancer lesions and their microenvironment
- Refining technology platforms for early detection of cancer biomarkers ex vivo
- Engineering nanoparticles to avoid biological and biophysical barriers.

Nanotechnology in Cancer Treatment

The use of nanotechnology in cancer treatment offers some exciting possibilities, including the possibility of destroying cancer tumors with minimal damage to healthy tissue and organs, as well as the detection and elimination of cancer cells before they form tumors. Most efforts to improve cancer treatment through nanotechnology are at the research or development stage. However the effort to make these treatments a reality is highly focused. For example, The Alliance for Nanotechnology in Cancer, established by the U.S. National Cancer Institute, is fostering innovation and collaboration among researchers to resolve some of the major challenges in the application of nanotechnology to cancer. In addition, there are many universities and companies' worldwide working in this area. It is possible that these efforts will result in cancer becoming being nearly eliminated in a decade or so, in the same way that vaccines nearly eliminated smallpox in the last century. The next section provides examples of the research underway, a few of the methods discussed have reached the pre-clinical or clinical trial stage.

RESULTS

Energy-based Therapies

Some promising focus areas in energy-based therapy research are photodynamic, alternating magnetic field, microwave, radio frequency (RF), high intensity focused ultrasound (HIFU), and cryoablation therapies, each with their own advantages and disadvantages. An advantage of these methods over systemic treatments or surgical resection is a more localized destruction of diseased tissue while minimizing possible side effects such as systemic toxicity or infection. Also, these methods are considered minimally invasive and are primarily investigated as outpatient procedures. Energy-based

therapies destroy tumor cells by causing a local temperature excursion within the designated treatment area. Commonly, this procedure is applied through a minimally invasive probe insertion technique or the focusing of external high energy sources. Although the individual implementation of these thermal ablation methodologies are different depending on the energy source, the fundamental therapeutic mechanisms for these therapies can be divided into two categories, damage from heating to hyperthermic temperatures (usually > 43°C) or damage from cooling or freezing to cryothermic temperatures (usually < -20°C). The therapeutic benefit from both of these types of treatment are strongly temperature and time dependent with differing degrees of damage existing throughout a given treatment gradient, as shown in Figure 4.1 (left).

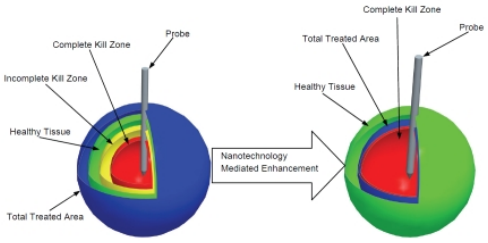

Figure 4.1: Illustration of nanotechnology mediated enhancements that can improve energy-based cancer therapies. Classical energy-based treatments have limitations in treatment area visualization causing the total treated area to overlap with healthy tissue. Also, unquantified thermal distribution causes an uncertainty in the complete kill zone (left). By using nanotechnology mediated combined modality treatments, the total treatment area can be visualized to minimize healthy tissue overlap, and the complete kill zone can be expanded to the treated area edge. In the complete kill zone, hyperthermic damage has been characterized by protein denaturation, cellular membrane damage, and vascular injury. Alternatively, cryothermic damage has been characterized by mechanical damage from ice formation, cellular dehydration, ischemia from vascular damage, and post treatment immunological response. Of the energy sources mentioned, all induce hyperthermic damage with the exception of cryoablation, which induces cryothermic damage. Although these methodologies have promising potential applications, they have problems that cannot be overlooked. Thermal ablation treatments are susceptible to uneven distribution of temperature profiles, and in the case of hyperthermic treatments, the treated area is not readily visible during the procedure and must be estimated from models or experimentation. Furthermore, the methods of implementation for the delivery of the thermal energy required for these treatments cause unintended damage to surrounding healthy tissue. In contrast, the iceball formed during cryothermic ablation treatment is visible through ultrasound or CT and easily tracked, but the determination of effectively treated area with temperature < -20 °C within the iceball is uncertain and must either be directly measured or estimated through models and experimentation. The fluctuation in temperature gradient and uncertainty in treated area causes ablation treatments to be less specific than intended and in some cases possibly incomplete, as shown in Figure 4.1 (left).

Adjuvants for Energy-based Therapy

To investigate enhancements to energy-based therapies, chemotherapeutic agents have been used as treatment adjuvants. It has been shown that both types of thermal ablation therapies, hyperthermic and cryothermic, have the potential to enhance the uptake of chemotherapeutic agents as well as induce a secondary immunological response that can enhance the extent of the removal of diseased tissue. Additionally, various salts, chemotherapeutic agents, and immunological factors have been tested for enhanced cryoablation treatment outcomes. While promising results and discoveries have been elucidated using adjuvants to enhance thermal ablation therapy, there are significant drawbacks associated with this treatment methodology that warrant further investigation. In particular, unquantified systemic toxicity, tumor specific targeting, and intratumoral drug distribution have left areas for improvements and research. The focus of further research in this field has been to improve the treated area versus non-treated area by using nanotechnology as a resolution enhancing mechanism to expand the complete kill zone into the incomplete kill zone, sharply define treatment boundaries, and reduce the total treated area, as shown in Figure 4.1 (right).

Nanotechnology Mediated Enhancements to Energy-based Therapies

Over the past decade, nanotechnology has begun to be explored as a tool to increase the resolution of thermal ablation treatment area, tumor visualization, and improve treatment effectiveness. The most direct method used for the enhancement of thermal therapy has been the systemic or local introduction of nanoparticles given concurrently with energy-based ablation treatments. For hyperthermic therapies,

carbon nanotubes, gold nanoshells, and iron oxide nanoparticles have proven extremely useful for enhancing heating effects due to energy absorption by the nanoparticles during treatment. Previous research has shown that the nanoparticles preferentially associate with tumors when given systemically or locally under the premise of the enhanced permeability and retention effect (EPR), which is often found in tumor vasculature. Furthermore, the use of metallic or carbon nanoparticles as treatment adjuvants enables the treated region to be visualized through noninvasive means such as MRI and CT, as shown in Figure 4.2.

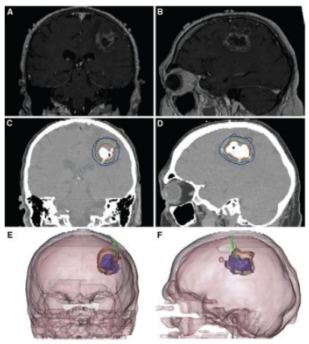

Figure 4.2: Imaging results from a preliminary study of locally delivered metallic nanoparticles in hyperthermic treatment of brain cancer: (**A,B**), pre-treatment brain MRI; (**C,D**), post nanoparticle delivery CT scan showing magnetic nanoparticle deposits as hyper-dense areas with the colored lines indicating calculated treatment temperatures between 40°C (blue) and 50°C (red) and the brown line representing the tumor area; and (E,F), 3-D reconstruction of fused MRI and CT showing the tumor (brown), magnetic fluid (blue) and thermometry catheter (green).

To overcome problems with systemic toxicity and enable target specificity, nanocapsule carriers with targeting moieties have been investigated to preferentially deliver therapeutics to diseased tissue via cell surface receptors, as shown in Figure 4.3. Some cell surface receptors help transmit messages from the extracellular environment to the intracellular environment, and in many cancerous cells are overexpressed. Overexpression of these surface receptors and other similar hallmarks specific to cancer can serve as potential target areas due to their increased concentration in diseased tissue. Specifically, receptors to estrogen, folic acid, epidermal growth factor and others have been explored for potential treatment targets. Using the various cellular targeting moieties, preferential uptake of nanoparticles into target expressing cancer cells has been shown.

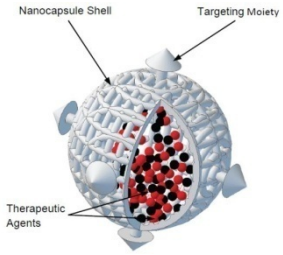

Figure 4.3: Illustration of nanocapsule containing therapeutic agents for targeted delivery to tumor cells. Nanoencapsulation technology has the potential to offer many combined modality approaches for treatment enhancements and personalized treatment targeting

In addition to small molecular compound drugs, a Nobel-prize winning discovery of RNA interference (RNAi) has been extensively applied with the progress of delivery systems in several different experimental models and more recently in treatment of numerous diseases, including neurodegenerative disorders and cancer. Small interfering RNAs (siRNAs) promote the cleavage of complementary mRNA to reduce protein production in mammalian cells and play a pivotal role in triggering RNAi. SiRNAs have short plasma half-life, fast degradation times in the physiological milieu, inefficient translocation into the cytoplasm, and lack of targeting ability. Therefore, successful siRNA-based gene targeting relies on the following conditions: improvement on stability and prevention of degradation by serum RNAses, efficient cellular uptake and subsequent intracellular release into the cytoplasm, as well as avoidance of intracellular immune responses, in vivo toxicity or rapid elimination in the liver or kidneys. SiRNA, similar to DNA, carries a net negative charge on the sugar phosphate which prevents its contact and entrance to the lipid bilayer of the cell membrane, whose head groups are also negatively charged. In the early 1970s, Calcium phosphate (CaP) precipitates were used as transfection reagents of viral DNA as they are believed to be non-toxic. CaP effectively protects the nucleic acids from enzymatic degradation

and aided cellular delivery, but uncontrollable rapid growth of calcium phosphate crystals greatly reduced the transfection efficiency. To facilitate higher genocom-patibility and lower toxicity, non-viral delivery vectors became a good choice for gene-based therapies and in drug development. Non-viral delivery vectors include cationic lipids (e.g. DO-TAP and Oligofectamine), cationic polymers (e.g. PEI and DAB dendrimers) and non-ionic (uncharged) polymers (e.g. poly HPMA and PEG). Nanoparticles (NPs) such as the cationic polymer, polyethyleneimine (PEI), can act as envelopes to protect the siRNA from metabolism and excretion, but can also carry specific molecules designed to target the siRNA to specific tissue types. For example, hydrophobic DOX obtained by deprotonation accumulated in the PCL core of the cationic micelle assembled from PEI-PCL, as shown in Figure 4.4. More recently, gold nanoparticles were directly conjugated to siRNA, increasing the serum half-life more than six fold compared to free RNA duplexes. Also, biodegradable nanoparticles have been developed and have shown good potential as carriers for anticancer drugs with a spherical structure. Within the past decade, the use of siRNA for RNAi has proven to be an effective nanomedicine for gene silencing therapy. However, research into the delivery of siRNA via nanoparticles to target cells is still in its infancy. In cancer therapy, siRNA delivery via nanoparticles needs to satisfy two major concerns: to improve the therapeutic range by including more than one siRNA which acts on specific targets, but keep minimal toxicity and maximum patient safety; and to develop novel or modify established carrier systems to induce gene changes on siRNA mediated gene silencing, but avoid enhancing the off-target gene changes. These emerging different new types of nanoparticles (biodegradable, gold, etc.) will facilitate the brilliance of RNAi and promote its application in clinical trials targeting specific tissues and diseases. Recently, thermally responsive nanoencapsulation systems have been developed using temperature sensitive carriers designed to deliver chemotherapeutic agents preferentially to tumor sites. During the temperature change associated with energy-based treatment, a conformational or structural change in the delivery vehicle causes the release of chemotherapeutics from the carrier. Once released, therapeutic agents are free to diffuse away from their carrier and act on nearby targets with promising results. Additionally, as previously mentioned, the solubility of many chemotherapeutic substances in physiological conditions is very poor. Therefore, an added benefit of nanoencapsulation is the expansion of available chemotherapeutic agents that can be used for treatment. Moreover, an effect of energy-based treatments is enhanced uptake of chemotherapeutics possibly due to permeability changes. Utilizing the nanoparticle aided target delivery approach allows drugs released via a temperature controlled mechanism to be preferentially distributed at the tumor location with an increased uptake caused from the energy-based treatment. Use of nanoencapsulation technology also has the potential to reduce systemic toxicity because of localized delivery of agents to the treatment area for controlled release. Consequently, this combined treatment has the potential benefit of reducing the overall treatment area by allowing for an increase in the complete kill zone aided by chemotherapeutic agents. Preliminary results in animal studies for temperature sensitive carriers (liposomes) have prompted several currently ongoing clinical trials in various phases, I-III.

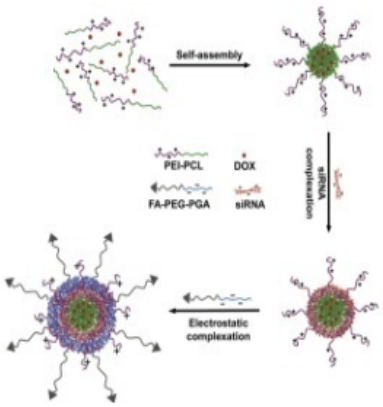

Figure 4.4: Formation of hierarchical nano-assemblies for combinatorial delivery of siRNA and anticancer drugs

To provide additional improvements to the nanoparticle aided delivery methodology, facilitated drug release and treatment visualization, some experimental systems have co-encapsulated metallic nanoparticles alongside chemotherapeutic agents. This co-encapsulation paradigm allows metallic nanoparticles to act as agents for imaging and controlled release of chemotherapeutics through their energy absorbing properties. Moreover, the delivery of metallic nanoparticles and chemotherapeutic agents simultaneously provide an approximated visualization of drug delivery localization and treatment area.

Therefore, this combined approach has the potential to reduce the total treatment area due to the energy absorbing properties of metallic nanoparticles, provide an increase to the complete kill zone from both targeted heating and chemotherapeutic agent delivery, and visual definition of treatment boundaries. However, more research into the development of this approach is necessary for clinical application to be realized, especially in the area of intratumoral nanoparticle distribution. Exploration into immune response to enhance treatment has also been studied by conjugating TNF-α onto the surface of gold nanoparticles. Results from animal model studies have shown a preferential biodistribution of gold -TNF-α within tumor locations with less systemic toxicity than free TNF-α. Furthermore, hyperthermic and cryothermic ablation treatment given after gold-TNF-α nanoparticle delivery increased the complete kill zone in animal models, as shown in Figure 5. From these initial studies, further tumor model applications and combinations with chemotherapeutic and co-encapsulation treatments are warranted.

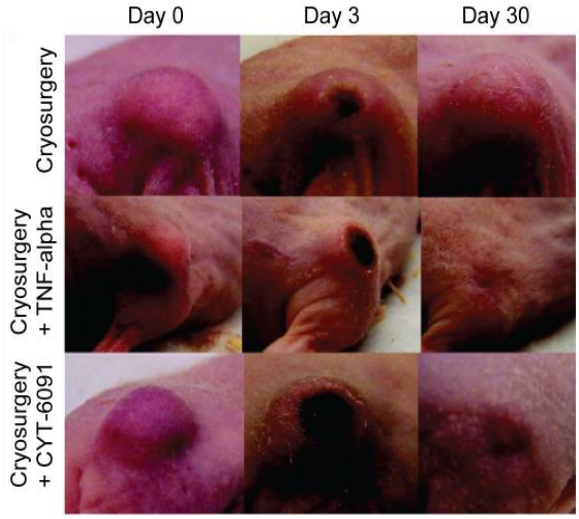

Figure 4.5: 30 day observation of tumor size in mice treated with cryosurgery, TNF-α with cryosurgery, or gold-TNF-α nanoparticles (CYT-6091) with cryosurgery. CYT-6091 was found to have less systemic toxicity than free TNF-α and provided a similar benefit in tumor size reduction as the more toxic free TNF-α

In addition to the nanoparticle mediated combined modality treatments, recent developments such as nanoscissor technology in conjunction with gene and gene product specific targeting and manipulation may bring about new areas of research focus for even more combined modality therapies with patient specific cancer targeting treatments. Specifically, targeted DNA sequences have been manipulated through localized disruption by the utilization of the energy absorption properties of gold nanoparticles. Furthermore, gold and polymeric nanoparticles have also been used for DNA/ oligonucleotide conjugation to regulate transcription and translation in cell models. Considering that this research has used energy absorption, metallic nanoparticles, and targeted delivery techniques similar to that used in previously mentioned research areas, it is not a far stretch to imagine that combined therapy applications with the correction or elimination of damaged DNA or initiation of apoptotic signaling through nanomanipulation techniques may be of future relevance. These techniques are still in their infancy and much more research and technical advancement is needed in order for this to become a practical and economic reality. However, the pace of advancement toward affordable and accessible gene research technology for potential treatment personalization applications is increasing rapidly. While the majority of the advances made for nanotechnology derived delivery vehicles have been in the area of hyperthermic treatment, recent studies in our laboratories have focused on advancing cryoablation treatment using hypothermically responsive nanocapsules. The goal of this research has been to improve the effectiveness of cryoablation treatment by moving the complete kill zone closer to the edge of the ice ball (the total treated area) by releasing drugs from a nanocapsule carrier within the incomplete kill zone. If successful, the subsequent outcome of this treatment enhancement would yield a smaller ice ball needed to achieve a greater clinical response and thus less peripheral damage to adjacent tissues. As shown in Figure 6, our initial studies have shown that a thermally responsive nanocapsule system for delivery of chemotherapeutic agents during cryothermic ablation treatment is theoretically possible and further research in this area is warranted and ongoing.

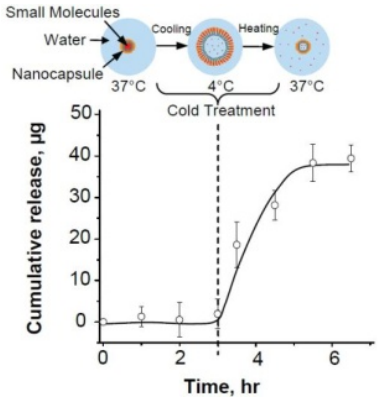

Figure 4.6: Cumulative release of ethidium bromide(EB) encapsulated in hypothermic temperature sensitive nanocapsules during dialysis. Before cold treatment, the nanocapsule solution was kept at 37°C to keep the encapsulated EB trapped within the capsule core to prevent release. After 3 hours, a cold treatment was given for 15 minutes to expand the nanocapsules and increase the wall permeability to allow the encapsulated EB to be liberated (released) out of the nanocapsule. As a result, a burst release of EB during dialysis is observable after the cold treatment

The potential benefits offered by nanotechnology (target specificity, reduction of systemic toxicity for chemotherapeutics, and coencapsulation of adjuvants), bring nanoparticle mediated combined therapies to the forefront of potential enhancements to energy-based cancer therapies. Coupled with further understanding of host immune response and the possibility of patient specific treatments, nanoparticle mediated therapies can also provide the basis for many more interesting and novel treatment options previously not investigated. Further research into the nanoparticle mediated enhancements to energy-based therapies mentioned in this review should result in the final goal of expanding the complete kill zone while minimizing the total treatment area (or incomplete kill zone) and providing visualization of boundary zones needed to give energy-based therapies more clinical relevance and certainty, as shown in Figure 4.1 (right).

CONCLUSION

In the past decades, the use of nanotechnology for drug delivery systems has grown exponentially. On the basis of considerable advances in the fabrication of drug nanocarriers with organic and/or inorganic architecture, worldwide pre-clinical researches have been underway with the current understanding of cancer, which undoubtedly expanded more than ever before. This review focused on drug delivery systems in cancer treatment using nanotechnology, providing an overview of the physicochemical principles of fine delivery systems targeted for cancer and cancer environments, whose pathophysiological characteristics are the strategic gateway for efficient nanoscale therapy. Ongoing developments have further expanded the boundary of this paradigm in medicine, such as the concept of “theranosis”, a system that can be used to perform diagnosis and therapy simultaneously. Although there have been toxicity and safety issues, we believe that we will benefit from the new knowledge of molecular events in cancer gathered by nanoscale drug delivery systems. With the continued discovery of new materials, the establishment of improved designs and considerate efforts for sophisticated optimization, we predict that a “cancer-overcoming era” will emerge. Through advanced understanding and application of functional nanomaterials, cellular targeting, and immune response, improvements in energy-based therapy have seen promising results. Furthermore, as gene sequencing technology advances to a much more affordable level, the personalization of nanotechnology derived delivery vehicles and therapeutics will make nanoparticle mediated combined therapies a much more focused and patient specific treatment option. Through further investigation and clinical trials, energy-based therapy assisted by nanotechnology may bring about a paradigm shift in primary cancer treatment in the not so distant future. Nanotechnology is considered one of the greatest man-made engineering marvels in minuscule scale. The technology has grown exponentially in recent years, and it arguably has had the most impact on contemporary science and society since technologies of the Industrial Revolution. Demand for this cutting-edge technology in biomedical fields is growing by more than 17% annually, and is expected to reach approximately $53 billion by 2011. One prospective report predicted that in the near future half of pharmaceutical industry products will have some connection with nanotechnology. Nanotechnology has already made an impact on cancer detection and therapy. The rapid intrusion of this cutting-edge technology in the current pharmaceutical industry is manifested by Abraxane, a nanomedicine approach to treat metastasis breast cancer. These aluminum-bound paclitaxel nanoparticles also have treatment

potential for other cancers with or without the co-presence of other anticancer drugs. Many nanomaterials like SPIO and USPIO nanoparticles are extensively used under various trademarks for imaging of various types of cancers. On the website ClinicalTrials.gov, a registry of federally and privately supported clinical trials conducted in the US and around the world, it is revealed that over 70 nanomedicine approaches are currently in clinical trials for cancer treatment and imaging. Though many of the technologies involving nanoparticles for cancer detection and treatment are mainly in preclinical stages, there is tremendous potential for nanotechnology to enable desperately-needed cancer detection in its early stages. Nano-carriers loaded with a chemotherapeutic payload targeting the tumor site can not only eliminate adverse side effects, but may also pave the way for bringing a more effective, specific, and personalized medicine for eradicating cancer and many other complex diseases. Thus, n nanotechnolgy has multifunctional proficiency and enormous potential to detect, treat, and monitor in real time. Nanotechnology applications in cancer detection and treatment have the potential to replace highly invasive conventional cancer detection and treatment, which often includes biopsies, irradiation, and painful therapies; they can become part of a painful past.

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