Engineered Nanoparticles in Cancer Therapy

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Abstract: Intense research has led to a more comprehensive understanding of cancer at the genetic, molecular, and cellular levels providing an avenue for methods of increasing antitumor efficacy of drugs while reducing systemic side effects. Nanoparticulate technology is of particular use in developing a new generation of more effective cancer therapies capable of overcoming the many biological, biophysical, and biomedical barriers that the body stages against a standard intervention. Nanoparticles show much promise in cancer therapy by selectively gaining access to tumor due to their small size and modifiability. Typically, though not exclusively, nanoparticles are defined as submicroscopic particles between 1 and 100 nm. Nanoparticles are formulated out of a variety of substances and engineered to carry an array of substances in a controlled and targeted manner. Nanoparticles are prepared to take advantage of fundamental cancer morphology and modes of development such as rapid proliferation of cells, antigen expression, and leaky tumor vasculature. In cancer treatment and detection nanoparticles serve many targeted functions in chemotherapy, radiotherapy, immunotherapy, immunodetection, thermotherapy, imaging, photodynamic therapy, and anti-angiogenesis. Not only are modifying agents allowing for greater and more accurate tumor targeting, they are also aiding in the crossing of biophysical barriers such as the blood brain barrier there by reducing peripheral effects and increasing the relative amount of drug reaching in the brain. Moreover, multifunctional nanoparticles perform many of these tasks simultaneously such as targeted delivery of a potent anticancer drug at the same time as an imaging material to visualize the effectiveness of the drug utilized for treatment follow-up. In this review, several recent US and World patents developing and modifying nanoparticles for the detection, analysis, and treatment of cancer are discussed.

Keywords: Nanoparticles, nanospheres, nanocapsules, nanotechnology, cancer, tumor, anticancer drugs, cytotoxic drugs, controlled release, targeted delivery, encapsulation, chemotherapy, radiotherapy, immunotherapy, thermotherapy.

INTRODUCTION

Typically, though not exclusively, nanoparticles are defined as microscopic particles between 1 nm and 100 nm, with some defining them up to 1 micron. Nanoparticulate delivery systems in cancer therapies provide better penetration of therapeutic and diagnostic substances within the body at a reduced risk in comparison to conventional cancer therapies. Nanoparticle distribution within the body is based on various parameters such as their relatively small size resulting in longer circulation times and their ability to take advantage of tumor characteristics. For example, nanoparticles less than 20 nm in size are able to pass through blood vessel walls and such small particle size allows for intravenous injection as well as intramuscular and subcutaneous applications. In comparison to conventional cancer treatments, the nanoscale of these particulate systems also minimizes the irritant reactions at the injection site. Nanoparticles can be formulated to exhibit stability, in both shelf storage life and uptake times, and can be designed for specific uptake within in the body and response by the body to treatment. Furthermore, nanoparticle size allows for interactions with biomolecules on the cell surfaces and within the cells in ways that do not necessarily alter the behavior and biochemical properties of those molecules [1].

Nanoparticles are formulated from a variety of materials and are engineered to carry an array of substances in a controlled and targeted manner. Nanoparticles are constructed to take advantage of fundamental cancer morphology and modes of development such as rapid proliferation of cells, antigen expression, and leaky tumor vasculature. Nanoparticles serving in anticancer therapies may be comprised, in whole or in part, of various lipids and natural and synthetic polymers. Most commonly used synthetic polymers to prepare nanoparticles for drug delivery are biodegradable. With the advances in polymer science, the field of polymeric nanoparticles has been expanding and gaining significant interest in recent years. A thorough discussion of polymeric nanoparticles is beyond the scope of this review, but the topic has been recently reviewed elsewhere [2]. Nanoparticles may also be composed of or transport a variety of substances such as silica, gold or other heavy metals, medicaments, quantum dots, nanocrystals, quantum rods, and various contrast agents. Surface properties' modifications allow for greater and more accurate tumor as well as conferring advantageous properties to the particle, such as increased solubility and biocompatibility useful in the crossing of biophysical barriers like the blood-brain barrier. Nanoparticulate surface modifications include coating/linking with folate, antibodies, adjuvants, ligands, antigens, proteins, enzymes, pH sensitive agents, and a plethora of other substances. In transportation, nanoparticles are prepared to prevent degradation of the carried load and protect transported substances from contact with healthy tissues thereby reducing peripheral effects and increasing the relative amount of the load reaching the diseased tissue. In this review, several recent US and World patents developing

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and modifying nanoparticles for the detection, analysis, and treatment of cancer are discussed. A summary of these patents' invention are listed in Table **1**.

CHEMOTHERAPY

Chemotherapeutic agents are cytotoxic drugs used to treat cancer that function by targeting fast growing cells and by blocking some critical element of the cell division process impairing mitosis as well as promoting apoptosis. Four major chemotherapeutic agents discussed in this review are plant alkaloids, antimetabolites, antitumor antibiotics and, the most commonly utilized type, alkylating agents. Oncologists administering conventional chemotherapy must balance drug dosage with the severity of side effects [3]. Successful chemotherapy of cancer depends on the delivery of sufficient concentrations of an effective drug to tumor cells without causing intolerable toxicity to the patient [4].

Nanoparticles and their use in drug delivery is a far more effective antitumor method than conventional chemotherapy, which is typically limited by the toxicity of drugs to normal tissues, short circulation half-life in plasma, limited aqueous solubility, and non-selectivity restricting therapeutic efficacy [5]. Nanoparticulate drug delivery systems are being developed to deliver smaller doses of chemotherapeutic agents in an effective form and control drug distribution within the body. Nanoparticles useful in transportation of anticancer drugs may consist of polymeric matrixes or of a reservoir system in which an oily or aqueous core is surrounded by a thin polymeric wall. Other reservoir systems can also be formed from natural macromolecules, non polar lipids, and inorganic materials such as silica. Transported chemotherapeutic agents that are lipophilic in nature may have some solubility either in the polymeric matrix or in the oily core of a reservoir system while hydrophilic agents may be adsorbed onto the particle surface [6]. Micelles can be utilized to entrap hydrophobic drug compounds within

Table 1. Summary of the Patents Invention

Application	Summary of Invention	References
Drug delivery, targeting	Nanoparticles containing paclitaxel formed by solvent evaporation from oil in water emulsion coated with human serum albumin	[10]
Drug delivery	Liposomes encapsulating taxol formed by high pressure homogenization	[11]
Drug delivery	Water-soluble chitosan containing paclitaxel formed by combining methoxy poly (ethylene glycol) *p*-nitrophenyl carbonate and cholesteryl chloroformate with the free amine group of a water-soluble chitosan and enveloping the paclitaxel in cholesterol	[12]
Drug delivery, targeting	Gelatin PLGA nanoparticles containing paclitaxel coated with bioadhesive molecules	[4]
Drug delivery	Nanoparticles/liposomes containing Homoharringtonine, Curcumol, Elemene, or Camptothecin and sterically stabilized polysaccharides of kelp as polymer	[13]
Drug delivery	Liposomes encapsulating cisplatin with the addition of membrane fusion peptides/molecules with fusogenic properties	[16]
Drug delivery	Nanoparticles containing methotrexate formed from water-in-oil emulsion utilizing high-pressure homogenization	[17]
Drug delivery	Polymeric nanoparticles containing doxorubicin coated with Tween R80 (Polysorbate)	[18]
Targeting, drug delivery	Hollow protein nanoparticles containing ganciclovir encapsulating thymidine kinase (HSV1tk) modified to display a hepatitis B virus surface-antigen for hepatocyte recognition	[21]
Targeting, drug delivery	Hollow protein nanoparticles containing ganciclovir encapsulating thymidine kinase (HSV1tk) modified to express an antibody that recognizes an epidermal growth factor receptor	[22]
Targeting	Gold nanoparticles conjugated to Thomsen-Friedenreich disaccharide antigen	[23]
Gene therapy, targeting	Nanoparticles formed from self-assembled aggregates of amphipathic molecules covalently linked to LM609 antibody and complexed with the plasmid	[25]
Gene therapy	Nanoparticle containing compacted vector formed by successive additions of oppositely charged polyelectrolytes including an incorporation of ligands into the DNA-polyelectrolyte shells which were mixed with Pluronic F127 gel and polyethylenimine	[26]
Vaccine therapy, targeting	Nanoparticles/liposomes containing epidermal growth factor receptor vaccine such as the mannan-modified nanoparticle, including mannan-modified recombinant adenoviral EGFR vaccine and protein vaccine, mannan-modified liposome recombinant EGFR gene vaccine and protein vaccine	[27]

(Table 1) Contd….

Application	Summary of Invention	References
Vaccine therapy, targeting	Nano vaccines prepared by envelopment through a magnetic ultrasonic process of an MG7-Ag analog epitope polypeptide and CpG ODN from a biological a nano emulsion, a gastric cancer antigen MG7, and a CpG sequence motif containing oligonucleotides serving as an immune adjuvant	[28]
Vaccine therapy, targeting	Nano vaccines/liposomes utilizing MAGE-1 and Hsp70 combined to form a fusion gene. The fusion protein and superantigen Staphylococcal enterotoxin A were combined to form a complex antigenic compound and encapsulated by a nanpliposome	[29]
Radiosensitizer, targeting	Gold nanoparticles coated with a mixture of alkanethiol and trimethylammonium thiol ligands	[30]
Radiosensitizer, targeting	Gold nanoparticles coated with thioglucose molecules and attached to anti-epidermal growth factor receptor antibody	[34]
Radiotherapy, imaging	Magnetic nanoparticles composed of CuxFe1-xO coated with surfactant utilizing decanoic acid and subsequently nonanoic acid	[35]
Radiotherapy, targeting, imaging	Carbon nanoparticles modified for antibody-antigen targeting utilizing fluorescein isothiocyanate-dextran for imaging. Cavitation induced by ultrasonic waves or local heating of the particles by pulsed electromagnetic radiation	[36]
Radiotherapy, targeting	Metal sulfide (PbS, ReS_2, and In_2S_3) nanoparticles designed to replace metal chelates in radioimmunoconjugates	[37]
Thermotherapy, targeting	Fe_3O_4 nanoparticles were surrounded by a dextran shell, to which the monoclonal antibody for Her-2 was covalently linked	[38]
Thermotherapy	Gold and iron nanoparticles served as radio frequency absorption enhancers	[39]
Photodynamic light therapy, targeting	Light-emitting DdSe quantum dots with an attached antibody	[40]
Drug delivery	Nanospheres containing paclitaxel, doxorubicin, or fluorodeoxyuridine formed by heating wax and dispersing drug into the melt	[42]
Targeting, imaging	Ferric oxide nanoparticles prepared by a reverse micelle colloidal reaction with two-photon dye ASPI-SH attached to the surface surrounded by a silica shell grown by tetraethylorthosilicate hydrolysis with targeting agent LH-RH coupled to the shell	[44]
Targeting, drug delivery, imaging	Nanoparticles containing paclitaxel formed from poly(-γ-glutamic acid)-poly(lactide) block copolymers loaded with drug using an emulsion/solvent evaporation technique conjugated with galactosamine targeting ASGP receptors and encapsulating a rhodamine-123 probe	[46]
Antiangiogenesis therapy, drug delivery, imaging	Nanoparticles composed of a core of HV sodium alginate, cellulose sulfate, luciferase or polymeric gadolinium, and anti-angiogenic factors, crosslinked with dextran polyaldehyde or conjugated to heparin sulfate surrounded by a corona of spermine hydrochloride, poly(methylene-co-guanidine) hydrochloride and pluronic F-68, calcium chloride, and a targeting ligand conjugated to polyethylene glycol or crosslinked to dextran polyaldehyde	[47]
Targeting, drug delivery	Nanoparticles/nanocapsules formed, for example, by coacervation of bovine serum albumin following desolvation, incorporated with antimitotic 5-fluorouracil, and coated with folate molecules	[48]
Thermotherapy, targeting, imaging	Nanoshells composed of a silica or a gold sulfide core and a gold shell doped with rare imaging earth ions	[49]
Thermotherapy, targeting, imaging	Nanoparticle clusters or agglomerates excited by an ultrasonic wave-generating source that provided lower intensity ultrasound radiation sufficient to produce and maintain microbubble “clouds”	[50]
Radiotherapy, targeting, imaging	Lu_2SiOs: Pr and $LuPO_4$: Bi nanoparticles conjugated to antibodies and excited by X-ray radiation leading to VUV or UV-C emission	[51]
Imaging	CdSe-ZnS quantum dots were incorporated with Texas Red-labeled dUTP enabling fluorescence energy transfer from excited particles to the dye and leading to a high fluorescence signal	[52]
Drug delivery, imaging	Liposomes formed by preparing a mixture of Spherex, containing carboplatinum, 5-fluorouracil, or 5-fluorouridine and iodine hydrogenated phosphatidylcholine, cholesterol, dicetyl phosphate and additionally polyethylene glycol in chloroform and diisopropyl ether	[53]

nanoparticles built up from polymers allowing drug delivery in effective concentrations of which would normally be insoluble in aqueous based formulations. In addition, nanoparticulate micelles enable more active drug to be delivered to their site of action by protecting the entrapped drug from hydrolysis by enzymes or from pH environments [7]. In certain cases, controlled nanoparticles provide a pre-programmed duration of action following a single dosage providing advantages over conventionally administered medicaments such as assured patient compliance with the dose regimen in addition to drug targeting to specific tissues or organs [8].

PLANT ALKALOIDS

Plant alkaloids are antitumor agents derived from plants whose function is blocking cell division during various phases by preventing microtubules from being synthesized. Paclitaxel, trade name Taxol, is a taxane that binds to the β subunit of tubulin promoting the polymerization of microtubuli from tubuli dimers and hyper-stabilizing microtubules by inhibiting their depolymerization. In addition, there is an abnormal arrangement and bundling of microtubuli during the entire cell cycle leading to the formation of multiple, microtubular dividing stars during mitosis. Thus the inhibition of the normal dynamic reorganization of the microtubular network results in preventing the restructuring of the cancer cell's cytoskeleton during cell division [9]. Paclitaxel has low solubility in water and the use of solubilizers is typically the culprit of anaphylactic reactions associated with paclitaxel based chemotherapeutic treatments [10]. A significant number of the patents in this review are focused on the nanoparticulate transportation of water insoluble plant alkaloid chemotherapeutic agents.

In an effort to improve the water solubility of paclitaxel, several investigators have modified its chemical structure with functional groups that impart enhanced water-solubility which may potentially induce undesired side-reactions such as allergic reactions and decrease the efficiency of the drug while adding to the cost of drug preparation. Desai *et al.* developed a method for the intravenous protein stabilized nanoparticulate delivery of paclitaxel in an unmodified form without causing allergic reactions. The nanoparticles less than 200 nm in diameter were created by a solvent evaporation technique from an oil in water emulsion containing paclitaxel with a coating of human serum albumin serving as a stabilizing agent. The combination of paclitaxel and albumin was preferred because of its low toxicity and non-myelosuppressive effects. Unlike conventional methods for nanoparticle formation, the addition of conventional surfactants, polymeric material, and foam suppressants were not required. Paclitaxel nanoparticles administered to tumor bearing mice showed a remarkable efficacy as all animals at the end of the study showed no evidence of recurrence and no evidence of tumors. A human clinical phase 1 trial showed a significant benefit to cancer patients [10].

Another method used to circumvent allergic reactions associated with taxols is to dissolve the nearly insoluble drug in the lipid phase of liposomes. Reszka *et al*. developed a method for the preparation of liposome-encapsulated taxol with a high taxol concentration and high stability prepared by high pressure homogenization. The liposomes can be designed for intravenous or intramuscular administration, and by aerosol formulation for inhalation administration. A human breast cancer bearing animal model utilizing the liposomes encapsulating taxol injected intravenously showed a better therapeutic effectiveness and a lower hemotoxicity than did free taxol [11].

Water-soluble chitosan may serve as an additional carrier to compensate for the insolubility of paclitaxel. Nah *et al.* produced a water-soluble chitosan nanoparticle by combining hydrophilic group methoxy poly (ethylene glycol) *p*-nitrophenyl carbonate and a hydrophobic group cholesteryl chloroformate with the free amine group of a water-soluble chitosan. Furthermore, paclitaxel was enveloped in cholesterol, a hydrophobic group of the above water-soluble chitosan nanoparticle thus producing the water-soluble chitosan paclitaxel containing nanoparticles. Water-soluble chitosan is stabilized better in weak acid, so that the nanoparticles of the invention target cancer cells rather than normal healthy cells, resulting in better accumulation of the nanoparticles in cancer cells. In addition, the water-soluble chitosan nanoparticles have many advantages of a polymer because it is produced based on a natural polymer. Antitumor efficacy was determined by the administration of a high dose of water-soluble chitosan paclitaxel containing nanoparticles to a tumor induced murine model resulting in a significant anticancer effect [12].

Nanoparticle carriers of encapsulated paclitaxel may also be modified to provide targeted delivery to various areas of the body. Au *et al*. developed a method of preparing paclitaxel-loaded gelatin poly(lactide-co-glycolide) nanoparticles for targeted drug delivery to tumors located in the peritoneal cavity, bladder tissues, and kidneys. The drug-loaded nanoparticles were modified by coating the gelatin framework with bioadhesive molecules to prolong the retention of these particles in the bladder cavity or bladder tissues beyond the treatment duration. Animal models showed that the nanoparticles released a significant fraction of the drug load within a 2-hour treatment interval resulting in a paclitaxel concentration in the tissue sufficient to produce antitumor activity in human bladder tumors. Paclitaxel released from the nanoparticles penetrated into the bladder and was found to be retained in the bladder tissues for periods extending beyond the 2-hour treatment duration. In addition, intravenous administration of gelatin nanoparticles resulted in increased localization of the drug contents in the kidney [4].

Polysaccharides of kelp (PK) possess innate anticancer properties such as inhibiting leukemia and solid tumors, and which increase immune function. Liu developed a technique utilizing PK as a polymer in the formation of drug containing nanoparticles and liposomes. The average diameter of the formed PK-drug nanoparticles was 80 nm and the PK-drug liposomes ranged in size from 20-50 nm in diameter. PK-drug nanoparticles and PK drug sterically stabilized liposomes formulated contained the following the anticancer drugs, Homoharringtonine, Curcumol, Eelemene, and Camptothecin. Murine model studies showed the therapeutic efficacy of each encapsulated drug was increased due to a delayed clearance from circulation, protection of the drug

from the biological environment, and reduction in drug uptake in healthy tissues in comparison to free drug [13].

ALKYLATING AGENTS

Alkylating agents are able to target tumor cells in various and multiple phases of the cell cycle and are better suited for the treatment of slow growing cancers. Alkylating agents stunt tumor growth by cross-linking guanine nucleobases resulting in abnormal base pairing or DNA strand breaks. Tumor DNA is unable to uncoil and separate which prevents the cell from dividing. Typically, alkylating agents act nonspecifically requiring conversion into active substances *in vivo* [14]. Cisplatin is one of the most widely used antineoplastic alkylating agents for the treatment of certain cancers such as testicular, ovarian carcinomas, and carcinomas of the head and neck [15]. Therapeutic efficiency of the delivery of cisplatin is limited by the low aqueous solubility and low stability of cisplatin and by the high toxicity of the drug.

Boulikas developed a method of reducing therapeutic difficulties associated with cisplatin preparation and use by encapsulating cisplatin into liposomes having a different lipid composition between the inner and outer membrane bilayers enabling cisplatin to reach primary tumors and their metastases after intravenous injection. The method involved a complex formation between cisplatin with dipalmitoyl phosphatidyl glycerol (DPPG) to convert cisplatin to its positively-charged active aqua form by hydrolysis. In addition, membrane fusion peptides and other molecules with fusogenic properties were added to improve entrance across the cell membrane of the complex. The aqua cisplatin-DPPG micelles were converted into liposomes 100-160 nm in diameter by mixing with vesicle forming lipids followed by dialysis and extrusion through membranes, entrapping and encapsulating cisplatin with a very high yield. Therapeutic efficacy was determined utilizing a human breast carcinoma MCF-7 bearing murine model. Significant MCF-7 tumor regression due to apoptosis was seen after intravenous injections of the liposome encapsulated cisplatin [16].

ANTIMETABOLITES

Antimetabolites are anti-cancer agents very similar to metabolites required for cellular biochemical reactions. Antimetabolites are cell cycle specific and are most effective as antitumor agents during the S-phase of cell division because they primarily act upon cells undergoing synthesis of new DNA for formation of new cells. Upon ingestion by cancer cells for use in the building of cellular DNA, the antimetabolites alter the function of enzymes required for cell metabolism and protein synthesis inhibiting the ability of a cell to produce or repair DNA, ultimately resulting in cellular death [14]. The antimetabolite methotrexate, trade names Rheumatrex and Trexall, competitively and reversibly inhibits dihydrofolate reductase which catalyses the conversion of dihydrofolate to the active tetrahydrofolate. Methotrexate, administered intrathecally or intraventricularly, may result in inflammation of the membrane surrounding the brain and spinal column [17].

Hassan *et al.* devised a process of forming nanoparticles containing substantially water insoluble methotrexate. The nanoparticles with a mean diameter of 198 nm were produced from a water-in-oil emulsion utilizing a high-pressure homogenizer by dissoliving methotrexate in an ammonium hydroxide aqueous solution and adding a mixture of sorbitan sesquioleate in triethylcitrate. Hassan *et al*. proposed the utilization of nanoparticles for intravascular injections for cancer treatment and/or diagnosis and extravascular injections to provide controlled release of the drug at the site of injection for prolonged drug effects with minimized multiple dosing [17].

ANTITUMOR ANTIBIOTICS

Antitumor antibiotics are anticancer agents capable of targeting tumor cells in various and multiple phases of the cell cycle and function by binding to DNA through intercalation between two adjacent nucleotide bases and by preventing the complex from separating, by inhibiting RNA preventing protein synthesis, and interfering with cell replication [14]. Doxorubicin, trade names Adriamycin and Rubex, is an anthracycline antitumor antibiotic used to treat primarily solid tumors that functions by intercalating with DNA which is believed to be mediated either by the enzyme DNA Topoisomerase II or by the formation of free radicals affecting many functions of the DNA, including DNA and RNA synthesis with possible breakage of the DNA strand occurring. Anthracyclines can also cause single-strand breakage as a result of the formation of active oxygen species. Cardiac toxicity, although rare, may occur with the risk of impaired heart function increasing with cumulative doxorubicin therapy due to the production of reactive oxygen species [9].

Anticancer agents known to have a high effect in the anticancer treatment, such as doxorubicin, either are unable to pass the blood brain barrier (BBB) or do not sufficiently pass in a effective amount and are effective only when delivered directly into the brain through a very complicated and sometimes risky surgery procedure. Sabel *et al.* has developed a method of preparing nanoparticles of polymeric material such as, but not limited to, polyacrylates, polymethacrylates, poly-cyanolacrylates, containing doxorubicin surrounded by a coating of Tween R80 (Polysorbate) for the transportation of doxorubicin directly to the brain by crossing the BBB. Efficacy was determined through intravenous administration of the doxorubicin nanoparticles in an animal model. Results clearly showed that the surfactant-coated nanoparticles were suitable to promote the passage of an effective dose of the anticancer agent doxorubicin across the BBB for a successful anticancer treatment [18].

IMMUNOTHERAPY

Immunotherapy is a form of treatment that stimulates the immune system, utilizing agents such as monoclonal antibodies, cytokines, or vaccines to attack tumor cells. Immunotherapeutic agents function in anticancer therapies by decreasing suppressor mechanisms, by stimulating the antitumor response due to increasing the number of effector cells, or by producing one or more soluble mediators. Immunotherapeutic agents may also function by altering tumor cells to increase their immunogenicity and by making them more susceptible to immunologic defenses or by increasing tolerance to cytotoxic drugs [19]. In immunotherapy, nanoparticle delivery systems utilize markers that

are expressed only by cancer cells or over-expressed by cancer cells relative to normal cells to target tumor cells and stimulate immunological responses in immunotherapy.

MONOCLONAL ANTIBODIES

Due to their genetic defects, many tumor cells display unusual antigens that are either inappropriate for the cell type, environment, or temporal placement in the organisms' development. The immune responses elicited by tumor antigens are not inherently strong because they are recognized as self cells given that tumor antigens are self antigens. To strengthen the immune response, highly specific monoclonal antibodies (mAbs) are used to intensify the immune system's antitumor capacity. The majority of mAbs are produced in large quantities by the clones of a single hybridoma cell which results from the fusion of an antibody producing myeloma and an antigenically-stimulated normal plasma cell to bind specifically to designated tumor cell antigens. Once mAbs are bound to tumor antigens, they can destroy cancer cells by directly inducing apoptosis, blocking growth factor receptors, inducing anti-idiotype formation and, indirectly, by activating complement-mediated cellular cytotoxicity and antibody dependent cell-mediated cytotoxicity. In addition, mAbs may serve as highly specific probes when they are attached to nanoparticulate vehicles to aid in the targeted delivery of transporting various agents such as antitumor cytostatic agents [20].

Monoclonal antibodies were utilized for targeting purposes in a method developed by Kuroda *et al*. for the preparation of hollow protein nanoparticles containing the drug ganciclovir encapsulating a hepatic cancer therapeutic gene, thymidine kinase (HSV1tk), derived from simple herpes virus. The nanoparticle was modified to possess hepatocyte recognition ability and particle formation ability by displaying a hepatitis B virus surface-antigen. A human hepatic cancer bearing animal model showed that when hepatitis B virus surface-antigen (HBsAg) particles encapsulating a hepatic cancer-treating gene were administered through intravenous injection, the gene was specifically incorporated into a human liver-derived tissue part. Cancer cell sensitivity to ganciclovir was induced through the incorporation and expression of the HSV1tk gene in the diseased tissues. Subsequent administration of ganciclovir effectively destroyed the cancer cells. Retraction of the human hepatic cancer-derived tumor cells over time was observed, and the therapeutic effect specific to hepatic cancer using the HBsAg-HSV1tk hollow protein nanoparticles was confirmed [21].

In a further exploration of the usefulness of hollow protein nanoparticles Kuroda *et al*. developed a method of encapsulating drug containing a cancer treating gene within nanoparticles modified to display an antibody used for specific targeting to human squamous carcinoma cells. As in the prior invention the nanoparticle transported the drug ganciclovir encapsulating thymidine kinase. However, the nanoparticle was modified to express an antibody that recognized the epidermal growth factor receptor, expressed by the cancer cells. Animal studies showed that the transfer and expression of the gene was very specific and efficient and therefore highly effective in human squamous carcinoma treatment [22].

CARBOHYDRATE-ANTIGEN BINDING

In addition to antibody-antigen binding, carbohydrate-antigen binding is useful in antitumor immunotherapy. Carbohydrates covalently linked to proteins or lipids are present on the surfaces of cells and have been shown to mediate important physiological phenomena such as cell-cell/cell-matrix communication and signal transduction. The biosynthesis of aberrant glycan chains due to changes in the expression of glycoprocessing enzymes is a characteristic of tumor formation. It is believed as these aberrations become more marked the tumor acquires a more aggressive phenotype. Many of these glycans are known as tumor-associated antigens and have been used as haptens in the development of tumor vaccines for target antitumor treatment. Two of the most widely distributed cancer-associated cell surface carbohydrate moieties are the tumor-associated Thomsen-Friedenreich (TF) disaccharide antigen and the (TN) nouvelle monosaccharide antigen which are shielded in healthy and benign-diseased tissues, but are uncovered in approximately 90% of carcinomas. TF-antigen (TF-Ag)-mediated tumor-endothelial cell interactions using synthetic compounds either mimic or mask the carbohydrate structure which can be utilized to inhibit tumor cell adhesion [23].

In the following example, Barchi *et al*. developed methods of preparing carbohydrate nanoparticle conjugates, wherein the carbohydrate antigens were specifically expressible on a tumor cell surface to reduce metastasis of carcinoma cells. A 4T1 murine breast cancer model was investigated using prepared TF-Ag conjugated gold nanoparticles injected intraperitoneally. The TF-Ag gold nanoparticle conjugates inhibited metastases from the metastatic site in the lungs. It was concluded that antibody to TF-Ag interactions apparently can either increase or decrease proliferation rate, as can lectins that bind TF-Ag. Furthermore, it was proposed that TF-Ag containing gold nanoparticle interferes with a process involved in replication of the tumor cell, as well as being able to inhibit adhesion [23].

NONVIRAL GENE THERAPY

Gene therapy is a method used to stimulate the body's immune response to attack cancer cells by introducing genetic material (DNA or RNA) to activate cellular processes for reducing or eliminating disease. Some forms of gene therapy function by preventing angiogenesis while others increase the sensitivity of cancer cells to other forms of cancer therapy such as chemotherapy and radiotherapy. Cancer cell death may be induced by introducing cancer cells with genes encoding apoptosis that are activated with the subsequent administration of pro-drug. The pro-drug is conjugated to a tumor specific molecule that remains inactive until it is signaled to activate upon reaching tumor cells [24]. Several gene transfer vectors such as viruses and liposomes have been utilized in gene therapy [25]. Methods of non-viral gene transfer are based on an interaction of the transgenic material with suitable carrier molecules resulting in transformation of the genetic material into a condensed conformational state enabling reception by the recipient cell, protection of the genetic material from degradation, and release from the carrier [26].

Bednarski *et al*. developed a method utilizing targeted cross-linked nanoparticles for *in vivo* gene delivery. The nanoparticles were self-assembled aggregates of amphipathic molecules stabilized by cross-linking. The LM609 antibody, which is specific for the integrin αvβ3, was covalently linked to the complex providing for selective delivery of the complex to endothelial cells. The antibody targeted cationic cross-linked nanoparticle was then complexed with the plasmid. A portion of the amphipathic molecules were cationic amphipathic molecules that served to form tight complexes with the nucleic acid, thereby condensing it and protecting it from nuclease degradation. High avidity of the nanoparticles was observed in a cell adhesion assay and these materials were used to target endothelial cells expressing the integrin αvβ3. An animal model was used to determine that the αvβ3-targeted nanoparticles delivered to blood vessels caused tumor regression based on their ability to promote apoptosis of the angiogenic endothelium. Pronounced tumor regressions were achieved by systemic delivery of the nanoparticle complex resulting in an anti-angiogenic effect that was targeted to the tumor vasculature [25].

The development of a non-viral method for *in vivo* gene transfer was designed by Boettger *et al*. The vector was packed into compact nanoparticles by successive additions of oppositely charged polyelectrolytes including an incorporation of ligands into the DNA-polyelectrolyte shells which were mixed with Pluronic F127 gel serving as a biodegradable adhesive to keep shells in contact with the targeted vessel. The assembled transfection system used polyethylenimine, an effective DNA carrier, as the DNA compacting agent. Animal models were used to determine *in vitro* transfection efficiency as a result of the direct injection of shells with a mean size between 100-200 nm into tumors. The formed nanoparticles showed high gene delivery efficiency and were able deeply to penetrate into tissues representing a significant progress in the development of non-viral gene delivery systems for *in vivo* gene transfer [26].

VACCINE IMMUNOTHERAPY

Vaccines are utilized to stimulate the immune system to initiate an immune response against specific target substances. Various types of vaccine may be used to treat cancer such as whole tumor cell vaccines, dendritic cell vaccines, idiotype vaccines, antigen/adjuvant vaccines, and viral vectors and DNA vaccines. Although the immunologic effect is not always sufficient to reverse the progression of cancer, vaccines are generally well tolerated and provide useful anticancer effects in some situations [27]. Antigen cancer vaccines are typically composed of a cancer-associated antigen not normally present on healthy cells, along with other components, i.e. adjuvants, utilized to stimulate the immune response against antigens resulting in the destruction of malignant cells without harming normal cells and in some cases, preventing recurrence [20].

Epidermal growth factor receptor (EFGR) is widely found on the surface of normal mammalian epithelial cell surfaces. Many cancer cells over-express EGFR which makes it an ideal candidate to serve as a target molecule in cancer therapy. Tian *et al*. provided a method utilizing nanoparticles for packaging antigens in therapeutic anti-tumor EGFR molecular vaccines used for targeting solid tumors expressing an xenogenic homologous EGFR molecule as the antigen. The mechanism of anti-tumor effects is that the EGFR molecule is an immune cross-reactive antigen, which can break the body's tolerance for self-EGFR molecules, and induce self cross-reactive immune responses against the EGFR molecule. The EGFR molecular vaccine has an effect on all the solid tumors wherein EGFR is over-expressed. Several methods for developing the EGFR molecular vaccine were performed including: (1) mannan-modified nanoparticle, including mannan-modified recombinant adenoviral EGFR vaccine and protein vaccine, mannan-modified liposome recombinant EGFR gene vaccine and protein vaccine, (2) gene-targeted nanoparticle, including gene-targeted nanoliposome recombinant EGFR vaccine, gene-targeted nano-PLGA recombinant EGFR vaccine, and gene-targeted recombinant adenoviral EGFR vaccine, which express EGFR and MIP-3 simultaneously, (3) recombinant adenoviral EGFR vaccine targeting cancer vascular endothelial cells, including RGD-modified recombinant adenoviral EGFR vaccine. Animal models were used to determine that an intramuscular administration of the EGFR molecular vaccine possessed effective anti-tumor functions, including preventing tumor formation, inhibiting tumor growth, exhibiting an anti-metastatic effect on tumors, and increasing the survival rate of animals carrying tumors. Specifically, it was found that EGFR molecular vaccine targeted nanoparticles increased the immunogenicity of EGFR greatly compared to a normal EGFR molecular vaccine, and thus enabled EGFR to elicit a stronger anti-tumor immune response [27].

Methods for preparing an antigen/adjuvant vaccine were developed by Wu *et al*. involving the envelopment through a magnetic ultrasonic process of an MG7-Ag analog epitope polypeptide and CpG ODN from a biological a nano emulsion, a gastric cancer antigen MG7, and a CpG sequence motif containing oligonucleotides serving as an immune adjuvant. Analyses showed that the nano vaccine had a grain size of 20-30 nm and possessed a relatively high enveloping rate and stability. Utilizing a murine model, it was shown that inoculated vaccine induced production of anti-MG7-Ag specific antibody and specific CTL secreted IFN-gamma and a superior antigen-adjuvant envelope group resulting in significantly smaller tumor volume [28].

Sui *et al*. developed a method of engineering a malignant tumor broad-spectrum gene nano vaccine. Initially, a tumor specific common antigen gene melanin tumor antigen-l (MAGE-1) and biological immunoadjuvant heat shock protein 70 (Hsp70) were combined to form a fusion gene (MAGE-1-Hsp70). The fusion protein and superantigen staphylococcal enterotoxin A were combined to form a complex antigenic compound. Furthermore, a nanoliposome was prepared to cover the complex antigenic compound, utilizing the molecular connection of N-hydroxysuccinimide ester and Hsp70 to form N-Hsp70, including into the outer layer of the nanoliposome phospholipids membrane. The MAGE-1Hsp70 fusion protein was found to have high activity [29].

RADIOTHERAPY

Radiotherapy is the use of ionizing radiation for the curative, palliative, and prophylactic treatment for almost every type of solid cancer. Normal healthy cells possess mechanisms for repairing DNA breakage, whereas rapidly reproducing cancer cells have a diminished capacity for repairing DNA breakage due to their undifferentiated and stem cell-like nature. Radiotherapy induced DNA damage is inherited through cell division thus accumulating damage to the cancer cells that causes them to reproduce more slowly resulting in shrinkage or total destruction of the tumor. Additional radiotherapy antitumor capabilities result from the production of free radicals that damage the DNA of cells [30]. The major source of toxicity of ionizing X-rays is thought to originate from the secondary species such as Auger electrons and radicals generated in aqueous solutions [31]. Auger electrons cause single- and double-strand breaks in DNA through direct interactions and they interact with water molecules to produce radicals that react to break the backbone of the DNA [29,30,32,33]. Nanoparticles serve as and transport various compositions in radiation therapy to increase the effectiveness of cancer treatment and imaging, for example, radiopharmaceuticals, radiosensitizers, radioprotectors, radioimmunodection agents, and radio-immunotherapy agents. Radioprotectors serve to protect normal tissues from the damaging effects of radiation whereas radiosensitizers function to condition the tumor cells to be more easily damaged by making DNA more susceptible to radiation or extending the life of free radicals produced by the radiation [34].

Nanoparticle radiosensitizers served as an important component in a novel X-ray therapy developed by Guo. The treatment, termed Nanoparticle Enhanced X-ray Therapy (NEXT), used targeted nano material radiosensitizers (NMRS) to enhance electromagnetic radiation absorption causing localized damage to DNA or other cellular structures for cancer therapy. The NMRS were comprised of spherical or near spherical pure gold nanoparticles ranging from 2 to 20 nanometers in diameters. The core was solid gold while the surface of the nanoparticles was covered with a mixture of alkanethiol and trimethylammonium thiol ligands. The alkanethiol functioned as a protective layer for the nanoparticles, and the trimethylammonium thiol ligands made the nanoparticles more soluble in water and targeted DNA through electrostatic interactions. In response to exposure from electromagnetic radiation, the NMRS emitted Auger electrons, generated radicals, and directly damaged DNA eventually leading to the death of the targeted cells. Experiments performed revealed a 100% enhancement of relaxation for nanoparticle-bound supercoiled DNA in aqueous solution after exposure to hard X-ray radiation. Guo proposed that NMRS can also be used as detection agents in conjunction with Computed Tomography or Computerized Axial Tomography to help in early diagnosis/treatment of diseases such as cancer [30].

Gold nanoparticles serving as radiosensitizers were designed and prepared by Hainfield *et al.* to enhance the dose and effectiveness of x-rays for promoting the shrinkage and/or elimination of target cancerous tissues without unacceptable damage to surrounding normal tissues or substantial toxicity. Gold nanoparticles 0.8-3 nm in diameter were coated with thioglucose molecules serving as an organic shell and attached to anti-epidermal growth factor receptor (αEGFr) antibody for specific targeting of cancerous tissue. The nanoparticles were injected intravenously into an animal model and were found to selectively accumulate in solid tumors and remain in high concentration in tumors for a significantly longer period than surrounding non-tumor tissue. Results showed that 86% of tumors were reduced in size and eventually disappeared when treated with the gold nanoparticles and radiation compared to 20% reduction in size of tumors receiving radiation alone. Further results indicated there was no acute toxicity at given doses. In addition, no serious long-term adverse effects were observed in any of the animals treated and the gold nanoparticle agents used and were well tolerated even at concentrations in the blood of up to 5% gold by weight [34].

Kim *et al.* developed a method of preparing radioactive magnetic fluids for the treatment and diagnosis of cancer. The magnetic nanoparticles of CuxFe1-xO were coated with surfactant first utilizing decanoic acid and subsequently utilizing nonanoic acid. The tightly bonded decanoic acid and nonanoic acid layers increased particle-particle repulsion and allowed homogeneous and stable dispersal of the magnetic nanoparticles in water. Also, the exposed carboxylic acid of the surfactant prevented the magnetic nanoparticles from being oxidized by air. The Cu^{2+} component of the radioactive magnetic fluids radiated beta ray and gamma rays. The beta radiation effectively serves to kill tumor cells, while the gamma radiation is easily imaged with a gamma camera. Kim *et al.* determined that the radioactive magnetic fluids could be used as a therapeutic drug or diagnostic reagent for cancer treatment and imaging [35].

Esenaliev developed a system utilizing the interaction of electromagnetic pulses or ultrasonic radiation with nanoparticles or microparticles for the enhancement of drug delivery in solid tumors. Targeting of the particles was accomplished through the attachment of antibodies directed against antigens in tumor vasculature for selective delivery to tumor blood vessel walls. Upon delivery, cavitation can be induced by ultrasonic waves or local heating of the particles by pulsed electromagnetic radiation resulting in perforation of tumor blood vessels, microconvection in the interstitium, and perforation of cancer cell membrane, and therefore providing enhanced delivery of macromolecular therapeutic agents from blood into cancer cells with minimal thermal and mechanical damage to normal tissues. *Ex vivo* animal tissue studies were performed to determine particle penetration due to laser radiation and sonication utilizing activated carbon particles 1 μm in diameter placed within various animal tissues. Fluorescein isothiocyanate-dextran was utilized to study penetration of macromolecules in the tissues. Particle penetration due to laser radiation in rat liver tissue up to 160 μm was recorded. The recorded penetration distance due to sonication was up to 160 μm in the liver, up to 30 μm in the kidney, and up to 150 μm in lung tissue. The ultrasound data demonstrated that the interaction of the particles with ultrasonic radiation-induced cavitation in resulted in penetration of particles and macromolecules into the interstitium [36].

A method for preparing stable metal sulfide nanoparticles for replacing metal chelates in radioimmunoconjugates was developed by Kotov. Stabilizers and conjugating agents such as *p*-thioaniline and *N*-hydroxysuccinimide ester of biotin were used in the preparation of PbS, ReS_2, and In_2S_3 nanoparticles. The numerous stabilizer molecules coating nanoparticles allowed for several identical covalent bridges to be formed in one conjugation step making the overall stability of the conjugate substantially higher. The process of covalently linking small peptides, monoclonal antibodies, and chemically constructed assemblies of antibody fragments to the nanoparticles served to enhance targeting activity. Nanoparticle-protein conjugates were formed through a simple conjugation reaction between nanoparticles stabilized with a thiol bearing -NH2 group, bovine serum albumin, and glutaroaldehide. Similar to proteins, Kotov determined that the nanoparticles could accommodate several biotin moieties and stated that this method was well suited for the preparation of monoclonal antibody conjugates and radiopharmaceuticals [37].

THERMOTHERAPY

Thermotherapy is a method of utilizing hyperthermia directed towards body tissues for the purpose of damaging protein and structures within cancerous cells and, in some cases, causing tumor cells to directly undergo apoptosis. Healthy cells are capable of surviving exposure to temperatures up to around 46.5˚C whereas irreversible damage to diseased cells occurs at temperatures in a range from approximately 40˚C to about 46˚C. During thermo-therapy, surrounding healthy cells are more readily able to dissipate heat and maintain a normal temperature while the targeted tumors experience difficulty in dissipating heat due to the disorganized and compact vascular structure that is indicative of tumors [38]. Nanoparticles are utilized in various aspects of hyperthermia-based treatments such as serving as the agents of thermotherapy, sensitizers, and are used for targeting purposes such as utilizing antibody enhanced targeting to increase efficacy and to reduce hypothermia-associated side effects.

Handy *et al.* developed a method of preparing targeted nanoparticles for the delivery of thermotherapy for cancer treatment with minimal invasion and short treatment periods. Prepared ferromagnetic nanoparticles were coated with the biocompatible material poly (methacrylic acid-co-hydroxy-ethylmethacrylate) using free-radical polymerization. Sodium bis-2-ethylhexyl sulfosuccinate acted as an ionic surfactant to form a stabilizing layer around the magnetic particles. Antibodies were covalently attached to the coated magnetic particles. A magnetic field was applied to inductively heat the thermotherapeutic magnetic composition containing single-domain magnetic particles attached to a target-specific ligand. A breast cancer cell animal model was used to determine the efficacy of the bioprobes in which 50 nm Fe_3O_4 particles were surrounded by a dextran shell, to which the monoclonal antibody for Her-2 was covalently linked. A colon cancer cell bearing animal model was used to determine selective tumoricidal activity. MUC-1 bioprobes were formed from 50 nm dextran coated Fe_3O_4 particles to show targeting utilizing the MUC-1 receptor in breast cancer. Bioprobe targeting was also performed using vascular endothelial growth factor receptor found on non-small cell lung cancer [38].

Targeted radio frequency (RF) absorption enhancers function by adding one or more artificial RF absorption frequencies to cells in a target area, thus permitting hyperthermia generating RF signals at specific frequencies to heat the targeted cells. Kanzius *et al*. developed techniques for investigating RF absorbing nanoparticles acting as enhancers composed of electrically conductive materials, such as gold and iron to induce hyperthermia in cancerous tissue. Hyperthermia was induced utilizing a number of RF transmitters in circuit communication with various transmission head configurations transmitting RF signals through target areas to various reception head configurations in circuit communication with RF receivers. It was concluded by Kanzius *et al*. that a variety of transmitter circuits and transceiver circuits can be used with a large number of described RF absorption enhancing nanoparticles for RF-induced hyperthermia used for the treatment of cancer [39].

PHOTODYNAMIC THERAPY

Photodynamic therapy (PDT) is a form of cancer treatment that utilizes a photosensitizing agent and a fixed frequency laser light. Photosensitizers are injected into the blood stream and absorbed by cells throughout the body. The photosensitizing agent is absorbed by both healthy cells and cancer cells whereas the healthy cells are more efficient at eliminating the agent. After timing the treatment so that there will be a significant concentration of the photosensitizer in the target cancer cells, and not the healthy cells, the desired area is exposed to light having a wavelength or a waveband corresponding to a characteristic absorption wavelength of the photosensitive compound. The photosensitizer absorbs the light, consequently triggering the production of singlet oxygen and other highly reactive free radical species. This leads to a number of biological effects including damages to proteins, nucleic acids, lipids, and other cellular components, and often resulting in cell death and possible activation of the immune system to attack the tumor. PDT is mainly used to treat tumors on or just below the skin or on the lining of internal organs as the laser light currently in use cannot pass through more than approximately 3 centimeters of tissue and thus cannot treat tumors that have metastasized [40]. Nano-particles are currently being utilized for targeted transpor-tation of photosensitizers to increase the effectiveness of PDT against cancers.

Light-emitting (LE) nanoparticles serve to absorb light from a light source and re-emit light at a different wavelength activating nearby photodynamic drug (PD), thus treating the disease in any site where the PD drug and nanoparticles are located and where light from a light source can activate the LE nanoparticles. Chen developed a method of preparing compositions of light emitting nanoparticles, for example quantum dots, nanocrystals, quantum rods, and mixtures of these for the purpose of activating PD more effectively for use in photodynamic therapy. In one aspect of the experiment, solubility of LE nanoparticles was enhanced by coating DdSe quantum dots with ZnS attached to protein through a mercapto-acetic acid. Targeting of quantum dots was enhanced through the attachment of an antibody that

was bound to a specific blood vessel antigen produced by blood vessels within a tumor. PD and LE nanoparticles were covalently linked and attached to a polymeric backbone. Optical fiber composed of a biocompatible matrix with incorporated nanoparticles accompanied with an external LED or laser source providing light to the fiber was utilized. LE nanoparticles were incorporated into a polymeric sheath for insertion beneath a patient's skin using a removable needle in proximity to the treatment site. Furthermore, a total internal reflection (TIR) lens was incorporated into the sheath. The TIR lens served to capture light emitted by nanoparticles that would otherwise illuminate healthy tissue and to direct it to diseased tissue. Various methods utilizing photoluminescent nanoparticles to activate PD for photodynamic therapy were developed [40].

NANOPARTICULATE TARGETING

Nanoparticles may be delivered to specific sites by size-dependant passive targeting or by active targeting. Passive targeting is dependent on both tumor structure and the structure of surrounding inflamed tissues. Nanoparticulate delivery systems may exploit a characteristic of solid tumors such as the enhanced permeability and retention (EPR) effect in which tumor tissues display several distinctive characteristics such as hyper vasculature, defective vascular architecture and a deficient lymphatic drainage which leads macromolecules and particulates to be accumulated preferentially and to be retained for a longer time in tumors (Fig. **1**). Active targeting has been performed to obtain a high degree of selectivity to specific tissues and to enhance the uptake of nanoparticles into target areas such as cancer cells and angiogenic microcapillaries growing around malignant cells (Fig. **2**). Nanoparticles are modified to target inherent characteristics of cancer cells such as rapid proliferation and particular antigen presentation [41]. Nanoparticulate delivery systems utilizing specific targeting agents for cancer cells minimize the uptake of the anticancer agent by normal cells and enhance the entry and retention of the agent in tumor cells. These delivery systems include the anticancer agent, a targeting moiety-penetration enhancer, and a carrier. The types of molecules which are capable of specifically recognizing and binding to other biomolecules are receptors, receptor ligands, enzymes, and antibodies. In all cancer therapies, targeting through surface modification provides numerous avenues for increasing treatment specificity and accuracy while reducing toxicity to healthy cells.

Tumor targeting treatment can be enhanced by incorporating a controlled release system. Shefer *et al*. developed a method of preparing a controlled release system that utilizes solid hydrophobic nanospheres containing anticancer pharmaceutical drugs encapsulated in a pH sensitive microsphere

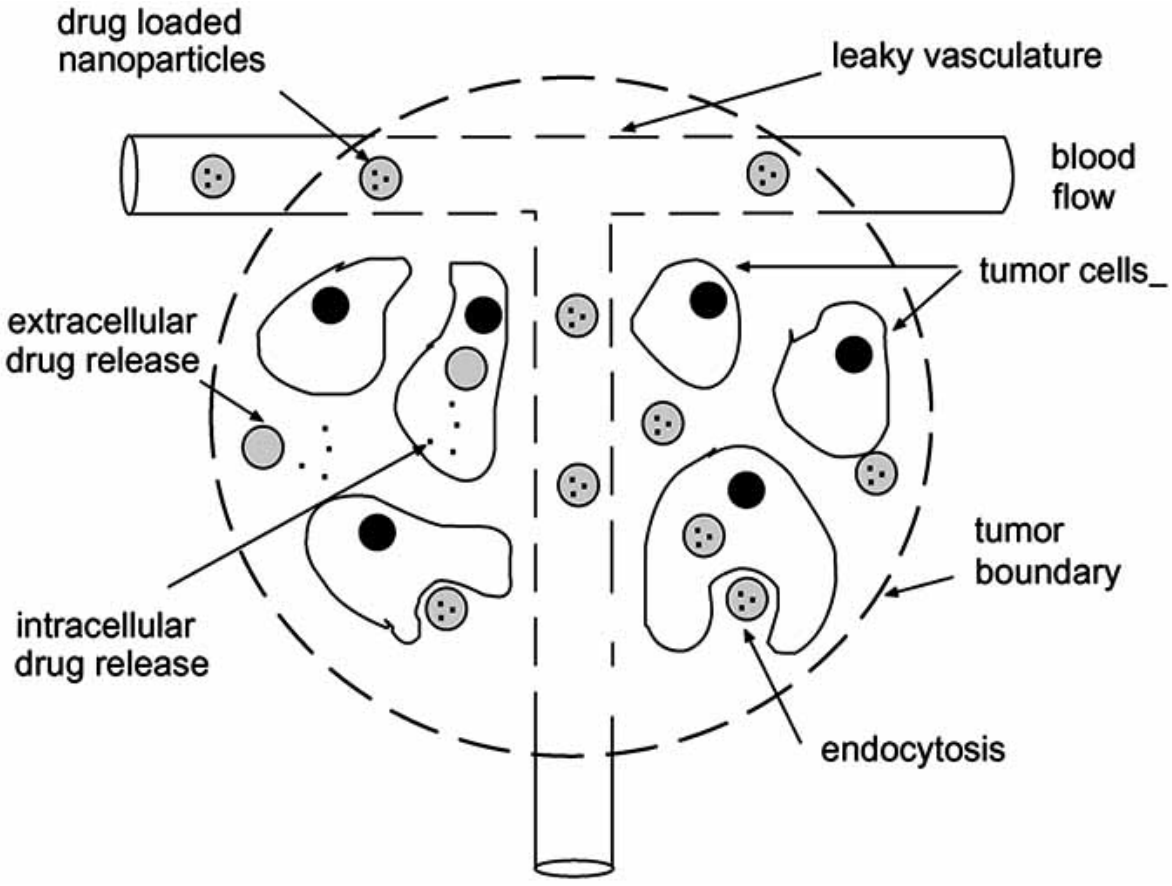

Fig. (1). Pasive targeting, the EPR effect. Tumor tissues are known to have leaky vasculature and results in a passive accumulation of nanoparticles and this phenomenon is referred to as EPR.

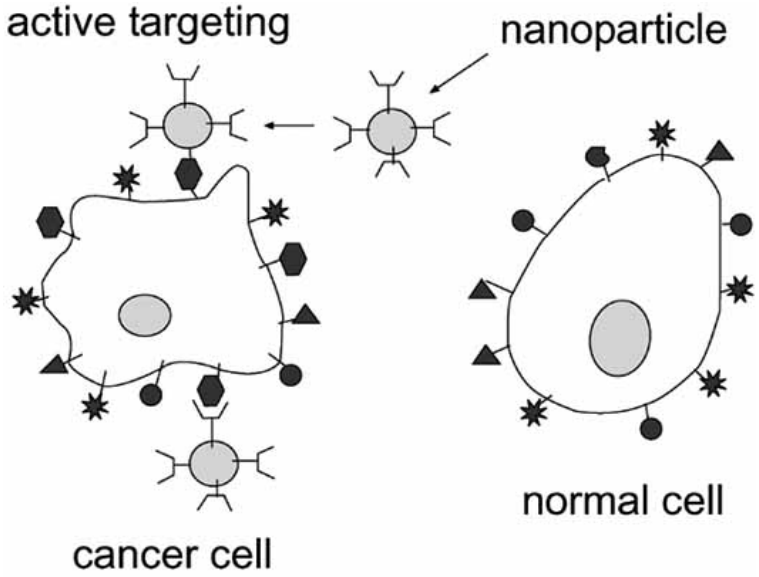

Fig. (2). Active targeting. Nanoparticles with ligands or molecules attached to their surface can target tumor cells preferentially over healthy cells.

for the treatment of cancer. Methods of targeting described included the addition of a bioadhesive material into the solid hydrophobic matrix of the nanospheres performed either by incorporating a bioadhesive material in the pH-sensitive microsphere matrix, or by using a bioadhesive material in the nanosphere matrix in conjunction with a bioadhesive material in the microsphere matrix. The nanosphere hydrophobic matrix was formed by heating candelilla wax and then dispersing paclitaxel into the melt. The microsphere pH sensitive matrix was created by adding the drug/wax mixture into an aqueous solution containing a pH dependent anionic polymer stable at pH 7.4 and solubilizing at pH 6 and lower. The suspension was spray dried to produce a free flowing, dry powder, consisting of 10% paclitaxel. In another example, doxorubicin was used as the chemotherapeutic drug. In an additional example, the nanosphere hydrophobic matrix was formed from bees' wax and the chemotherapeutic drug fluorodeoxyuridine. The nanospheres were designed to release the drug over an extended period of time by dissolving/swelling the microsphere at a pH that is typically found in or near cancerous tissue [42].

SURFACE RECEPTOR TARGETING

Many cancer cells overexpress luteinizing hormone-releasing hormone (LH-RH) receptors in their plasma membrane including ovarian, breast, and prostate cancers, though they are not detectably expressed in normal visceral organs, providing a target for certain anticancer therapies [43]. Prasad *et al*. developed a method of targeting LH-RH receptor bearing cancer cells with ferric oxide nanoparticles prepared by a reverse micelle colloidal reaction [44]. In a reverse micelle, the hydrophilic groups are sequestered in the micelle core and the hydrophobic groups remain solvent-exposed on the surface of the micelle and are formed from a surfactant, continuous oil phase and water [45]. A tracking agent two-photon dye, ASPI-SH, was attached to the surface of the iron oxide. Silica was added to pre-form the silica shell with additional silica shell grown by tetraethylorthosilicate hydrolysis. On the surface of the silica shell, the targeting agent LH-RH was coupled to the shell through carbon spacers so as to prevent stearic hindrance during the interaction of the targeting agent with its affinity molecule on cells. Following the administration of the nanoparticles to patients and the resultant internalization of the nanoparticles by the tumor cells, the patients were exposed to a DC magnetic field available in standard magnetic resonance imaging equipment. The selective interaction, internalization, and the effect of various conditions on the magnetocytolysis of cells of these nanoparticles were investigated by utilizing LH-RH receptor expressing cells on oral epithelial carcinoma cells. Data clearly showed that the nanoparticles selectively affected specific cell types with a controllable efficiency. The data also demonstrated the magnetocytolytic ability of these nanoparticles and that these effects required direct contact with and internalization into the target cell. The findings revealed magnetocytolytic activity was effective only in those cells capable of interacting with these nanoparticles and that the nanoparticles likely entered cells by receptor mediated endocytotic process. Furthermore, results showed that the lytic effect was dependent upon the time of exposure to the magnetic field [44].

Another receptor, asialoglycoprotein (ASGP), was utilized in nanoparticulate targeting of hepatoma cells for anticancer drug delivery. Sung *et al*. developed a method of preparing biodegradable nanoparticles with a mean size of 140 nm from poly(-γ-glutamic acid)-poly(lactide) block copolymers loaded with paclitaxel using an emulsion/solvent evaporation technique. The nanoparticles (NPs) were conjugated with galactosamine (GAL) via an amide linkage forming GAL-NPs to enhance hepatoma HepG2 cell uptake by targeting ASGP receptors located on their surfaces. Immunofluorescence analysis in a hepatoma-tumor-bearing murine model was performed utilizing a rhodamine-123 probe encapsulated in the hydrophobic core of the Gal-NPs. Anti-tumor efficacy of the Gal-NPs revealed that the active targeting nature of the Gal-NPs expressed a high degree of

selectivity to hepatic tumors with enhanced cellular uptake via receptor-mediated endocytosis, and that the subsequent release of the encapsulated paclitaxel inside the cytoplasm inhibited the growth of the cells resulting in reduced tumor size, with a consequent decrease in systemic toxicity compared to free paclitaxel. A dual-particle tumor targeting system was also described for selectively inhibiting angiogenesis within a hepatoma. The first component of the dual-particle tumor targeting system was nanoparticle encapsulating ganciclovir conjugated with galactosamine and the second component was an enhanced permeability and retention mediated targeting nanoparticle containing an HSV thymidine kinase (TK) gene. It was proposed that after cancer cells internalized the first and second nanoparticles together, thymidine kinase would digest ganciclovir to produce cytotoxic effects, thus killing the targeted cancer cell. The efficacy of the dual targeting system utilizing a radiograph was illustrated by encapsulating a radiotracer in liver tumor cells that expressed HSV-TK gene after up-taking both the first and the second nanoparticles [46].

ANGIOGENIC FACTOR TARGETING

Like receptor targeting, targeting of angiogenic factors also takes advantage of properties unique to cancer cells. Angiogenesis is a normal process in growth and development that involves the growth of new blood vessels from pre-existing vessels. In relation to cancers, angiogenesis may result in new blood vessel formations to serve diseased tissues as well as the destruction of normal tissues and tumor metastases. The body modulates angiogenesis by producing several angiogenesis-stimulating growth factors and several angiogenesis inhibitors. During tumor angiogenesis, cancerous cells produce abnormal amounts of angiogenic growth factors therefore overwhelming the effects of natural angiogenesis inhibitors. Anti-angiogenic treatment is the use of drugs or other substances to stop tumors from developing new blood vessels resulting in stunted tumor growth thereby avoiding tumor spread and the establishment of new distant metastases as well as promoting the shrinkage of tumors [9]. Several approaches are being investigated including the use of proteins, small molecules, gene therapies, and radiation therapies to disrupt tumor angiogenesis.

Prokop *et al*. provided methods for developing a series of biocompatible, nanoparticulate formulations used as drug delivery vehicles designed to retain and deliver anti-angiogenic compounds over an extended time course for targeting tumor vasculature. Nanoparticles were formulated comprising a water-based core of HV sodium alginate, cellulose sulfate, and anti-angiogenic factors such as thrombospondin (TSP) -1 or TSP-517, crosslinked with dextran polyaldehyde with calcium chloride or conjugated to heparin sulfate with sodium chloride. In addition bioluminescent agent, luciferase, or contrast agent, polymeric gadolinium, was located within the polyanionic core. The water-based corona surrounding the core was comprised of spermine hydrochloride, poly(methylene-co-guanidine) hydrochloride and pluronic F-68, calcium chloride, and a targeting ligand conjugated to an activated polyethylene glycol or crosslinked to dextran polyaldehyde. Biodistribution of targeted nanoparticles was evaluated by monitoring luciferase in a murine model. Biocompatibility and suppression of vascularization data collected indicated that normal angiogenesis due to wound healing was suppressed by means of the created anti-angiogenic factor nanoparticles. Animal survival studies of tumor bearing mice receiving a core-loaded TSP-1 and corona loaded TSP-521 peptide-PEG conjugate were performed. Noninvasive imaging was performed using luciferase and magnetic resonance imaging and gadolinium contrast agent loaded in a nanoparticle containing core-loaded TSP-1 and corona loaded TSP-521 peptide-PEG conjugate. Upon contrast agent equilibration, enhanced visualization of the tumor volume was perceived. A targeting experiment revealed that core loaded doxorubicin-polymer conjugate and corona loaded dextran/a-tetrasaccharide conjugate nanoparticles expressed to certain tumor cell tissue and cell lines. Furthermore, methods of producing nanoparticles as vehicles for anti-angiogenic compounds in batch or continuous mode via simple mixing or micromixing were provided [47].

FOLATE-MEDIATED TARGETING

An additional active targeting technique used in nanoparticle cancer therapies is folate-mediated targeting. An inherent characteristic of many cancer cells is the over expression of high-affinity folate receptors. Most human tissues lack the receptor, except the placenta, choroids plexus, lungs, and kidneys. Small nonimmunogenic stable folate has a high specificity for tumors. Folic acid enters tumor cells either through a carrier protein, a reduced folate carrier, or via receptor-mediated endocytosis facilitated by the folate receptor. Folate-mediated tumor targeting has been used to deliver the following substances to cancer cells such as protein toxins, low-molecular weight chemotherapeutic agents, radio-imaging agents, MRI contrast agents, radiotherapeutic agents, liposomes containing chemotherapeutic drugs, genes, antisense oligonucleotides, ribozymes, and immunotherapeutic agents [48].

Russell-Jones *et al*. developed methods of preparing folate-coated nanoparticles utilizing various techniques to amplify drug delivery. Nanoparticles were formed by coacervation of bovine serum albumin following desolvation, incorporated with antimitotic 5-fluorouracil, and coated with folate molecules by reaction of folate with 1-ethyl-3-(3-dimethylaminopropyl) carbodiomide and *N*-hydroxysuccinimide. The solvent evaporation technique was also utilized to prepare folate-phosphatidyl ethanolamine (PEA)-polymethylmethacrylate nanoparticles, folate-PEA-Poly-lactic acid nanoparticles, and folate-PEA-Poly-Hydroxy-butyrate/valerate nanoparticles. In another example, covalent conjugation of folate to nanoparticles was performed utilizing surface carboxyl groups as well as conjugation of folate to hydrazine modified poly-lactic acid nanoparticles. In addition, isobutyl-cyanoacrylate (IBCA) nanocapsules were prepared and coated with folate. The efficacy of the folate coated IBCA nanocapsules in folate-mediated targeting was determined using a tumor-bearing murine model that showed a significantly increased quantity of nanocapsules targeted to the tumor [48].

IMAGING

The most easily and effectively treated tumors are those that are in their earliest stages of development and are small

in size and localized. Conventional imaging technologies are not sensitive or accurate enough to detect the earliest tumor stages. Furthermore, most conventional techniques represent static images of events rather than a continuous visualization of tumor growth or death. Fortunately, nanoparticles are not only useful in functioning as anticancer therapy delivery vehicles, they can also be engineered to transport contrasting agents or serve as the imaging agents themselves. Nanoparticles can be engineered to serve as intense beacons for imaging purposes by carrying, for example, bioluminescent agents. Further modifications to enhance targeting provide avenues to ensure that a large concentration of imaging agents is located within the tumor and not the surrounding healthy tissues resulting in an increased signal and a reduced noise scenario. Nanoparticles also function in tumor imaging wherein tumor cells do not take up particles and create a "signal void" against a high proximal concentration of imaging materials [1]. Nanoparticulate imaging allows for the early detection of tumor and metastases as well as opportunities for real-time monitoring thereby increasing both sensitivity and accuracy of anticancer therapies. Contrast agents can be coupled with various anticancer therapies to monitor treatment successes and failures [49]. Imaging is an important tool for identification and three-dimensional location of diseased tissue and cells. Imaging can also indicate the location and boundaries of viable diseased cells or tissues during and after certain treatments, particularly in minimally invasive procedures. Agents of diagnostic imaging may be radio contrast agents, ultrasound contrast agents, magnetic contrast agents, etc. Typical diagnostic imaging methods used are ultrasound, MRI and X-ray [50].

West *et al*. developed a method of delivering targeted thermotherapy in combination with diagnostic imaging. Hypothermia was induced by delivering targeted nanoparticles 100 to 200 nm in diameter called nanoshells optimized for maximum absorption of electromagnetic radiation at a prescribed wavelength. The particles were composed of either a silica or a gold sulfide core and a gold shell. In the diagnostic portion of this patent, methods were described in which particles were prepared consisting of silica core doped with rare earth ions such as Neodymium 3+, Erbium 3+, and Praseodymium 3+ or a gold shell designed as either an absorber or a scatterer. These ions are of particular interest because they are robust infrared fluorophores and are used extensively as gain media in commercial near infrared solid state lasers and amplifiers. Rare earth ion incorporation was achieved by modifying the silica nanoparticle synthesis from basic to acidic conditions, under which the rare earth ions remain soluble and thus were incorporated into the nanoparticle. Praseodymium 3+ emission in bulk silica prepared by the standard high-temperature diffusion process was compared to a typical visible-region fluorescence spectrum of Praseodymium 3+. The rare earth doped silica nanoparticles showed utility as infrared fluorophores in bioimaging applications [49].

In an another example involving thermotherapy and imaging, Kislev described two types of systems, one type suitable for therapeutic treatment of cancer by inducing targeted hyperthermia and another type suitable for diagnostic applications providing precise imaging of the diseased tissue borders and volume. The difference between the types was the source of ultrasonic waves, wherein the therapeutic ultrasonic wave-generating source was designed to provide continuous, or pseudo continuous ultrasound radiation to be converted into heat. For the diagnostic application, an imaging ultrasonic wave-generating source provided lower intensity ultrasound radiation sufficient to produce and maintain microbubble "clouds" to serve as a contrast agent. When ultrasound waves encounter the microbubbles, changes in acoustic impedance produces a more intense reflection of sound waves and consequently a more intense signal in the ultrasound image. One example illustrated the guided treatment of a deep tissue in which nanoparticle clusters or agglomerates within the targeted tissue were exposed to the pulsed electromagnetic radiation generating a microbubble cloud which in turn was stabilized by the dispensed ultrasound radiation and converted a significant portion of the ultrasound radiation into heat emitted to the targeted tissue. An ultrasound probe sent ultrasound signals and received the ultrasound echo signals reflected through the targeted tissue. The echo signals were processed to generate an image of the targeted tissue during the treatment [50].

UV emitting nanoparticles may serve as an important component for early diagnosis of rapidly developing cancers as the potential higher detection sensitivity allows for improved medical imaging and possible early detection of diseased tissue. For therapeutic purposes, UV radiation is absorbed by the surrounding organic matrix, resulting in decomposition of the material. For imaging purposes, the endoscopic detection of the UV emission is used as a medical imaging technique to locate and study diseased tissue. Juestel *et al.* described techniques for using UV emitting nanoparticles for radiation therapy and medical imaging. Two illustrations were provided for the production of nanoparticles for radiation therapy. The nanoparticles were conjugated to antibodies used for specific binding of the complex to the cell membrane of cancer cells leading to a localized destruction of diseased tissue with a high efficacy and a lower level of destruction of surrounding healthy tissue. Lu_2SiOs: Pr nanoparticles were formulated and modified to form an aspartic acid/SiO_2 layer. Histidin-modified Bevacizumab was attached to the aspartic acid/SiO_2 layer by the formation of amide bridges. $LuPO_4$: Bi nanoparticles were formulated and modified to form an aspartic acid/dextran layer. Histidin-modified anti-CEA was attached to the aspartic acid/Dextran layer by the formation of amide bridges. Excitations X-ray radiation of the nanoparticles with high-energy radiation lead to VUV or UV-C emission. Juestel *et al*. concluded that due to the high sensitivity of the emitting nanoparticle to the exciting X-ray radiation, medical imaging sensitivity can increase using high X-ray dosage [51].

Patolsky *et al.* developed an optical method and device for the fast and sensitive detection of DNA or RNA analytes and DNA polymerase or telomerase analytes of cancer cells through telomerase activity and single-base mutations. The method and device was based on the use of semiconductor nanoparticles carrying a recognition agent, thus forming a hybrid system. Within this system, a reaction occurred causing the immobilization of an acceptor to the recognition

agent, either directly or through a reaction product of the recognition agent that was formed in the presence of the analyte. The nanoparticles provided an active media with respect to electromagnetic radiation. The detection was based on fluorescence energy transfer (FRET) between the active media semiconductor nanoparticle donors, which were excited with electromagnetic radiation, and acceptors, in the form of dye-labeled or semi-conductor nanoparticle-labeled agents that were immobilized to the recognition agent in the presence of the analyte. In one aspect of the investigation, CdSe-ZnS quantum dots were stabilized by a mercaptopropionic ligand, and modified with a thiolated oligonucleutide and incorporated with dye-labeled nucleotide, Texas Red-labeled dUTP, in the presence of telomerase. Upon excitation of the system, the incorporated dye units enabled FRET from excited particles to the dye and led to a high fluorescence signal characteristic to the dye for the observation of telomerization processes. FRET was also utilized for probing the dynamics of polymerase replication of DNA utilizing a primer complementary to M13 DNA hybridized to the CdSe-ZnS quantum dots with dNTPs containing a fluorophore-labeled nucleotide. Atomic Force Microscopy images illustrated the telomerization and replication processes [52].

Visualization of antitumor treatment efficacy was preformed by Reszka *et al*. with the development of a liposome containing the cytostatic agents Carboplatinum, 5-fluorouracil, or 5-fluorouridine and the contrast agent iodine encapsulated in SUV-polyethylene glycol (PEG) liposomes. The encapsulation of the cytostatic agents was performed by preparing a mixture of hydrogenated phosphatidylcholine, cholesterol, dicetyl phosphate and additionally polyethylene glycol in chloroform and diisopropyl ether. In addition, degradable starch, Spherex, was encapsulated to retard flow and increase contact time. Utilizing an animal model the area under the curve (AUC) value showing the residence time and the quantity of the therapeutic agent collected in the tumor was measured to determine the therapeutic effect. The AUC for liposomal Carboplatin and liposomal 5-FU was found to be increased in the tumor model by 20 times [53].

CURRENT & FUTURE DEVELOPMENTS

In this review we have explored nanoparticles serving as agents of novel antineoplastic treatments in various aspects of cancer therapies as well as imaging (Table 1). We have witnessed the use of nanoparticulate technology in developing a new generation of more effective cancer therapies capable of overcoming the many biological, biophysical, and biomedical barriers that the body stages against conventional cancer therapies. We have investigated techniques developed by talented scientists in the more widely utilized areas of cancer treatment such as chemotherapy, radiation, etc. and other areas such as photodynamic light therapy. Exciting new methods are being developed to formulate nanoparticles out of a seemingly endless number of compositions expressing an even greater number of functions in the fight against cancer. Their inherently small size and modifiability are allowing for innovative controlled and targeted techniques resulting in a drastic reduction in anticancer treatment side effects and increased antitumor efficacy. Combination nanoparticulate therapies have the potential to destroy cancers in their earliest stages in custom tailored treatments utilizing imaging to view treatment progress and success. Anticancer nanoparticulate technology is being developed with the goal to minimize side effects for nanoparticulate treatments by relying on nanoparticles that are perfectly engineered to attack cancer in a decisive manner with healthy tissues suffering no undesirable consequences at the initial stages of cancer cell development. Nanoparticles will likely serve as the norm rather than an exception in the majority of all areas of future conventional cancer treatments.

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REFERENCES

[1] McNeil SE. Nanotechnology for the biologist. J Leukoc Biol 2005; 78: 1-10.
[2] van Vlerken LE, Amiji MM. Multi-functional polymeric nanoparticles for tumour-targeted drug delivery. Expert Opin Drug Deliv 2006; 3: 205-16.
[3] Chemotherapy side effects. May 30, 2006. www.chemotherapy.com/side_effects/side_effects.jsp
[4] Au, J.L., Wientjes, M.G.: US2006034925A1 (**2006**).
[5] Li C. Poly(L-glutamic acid)–anticancer drug conjugates. Adv Drug Deliv Rev 2002; 54: 695-713.
[6] Kumar, S. Nanomaterials for Cancer Therapy. Weinheim, Germnay, Wiley-vch GmbH & Co. KHaA. 2006; 1-39.
[7] Micelle nanotechnology. October 20, 2006. http://www.maelor.plc.uk/mice.php
[8] Tice TR, Gilley RM. Preparation of injectable controlled-release microcapsules by a solvent-evaporation process. J Control Release 1985; 2: 343-52.
[9] Brannon-Peppas L, Blanchette JO. Nanoparticle and targeted systems for cancer therapy. Adv Drug Deliv Rev 2004; 56: 1649-59.
*[10] Desai, N.P., Tao, C., Yang, A., Louie, L., Yao, Z., Soon-Shiong, P., Magdassi, S.: US20046749868B1 (**2004**).
[11] Reszka, R., Brandl, M., Fichtner, I., Warnke, G.: US20006090955A (**2000**).
[12] Nah, J.W., Jung, T.R., Jang, M.K., Jeong, Y.I.: US2006013885A1 (**2006**).
[13] Liu, Y.: US2004192641 A1 (**2004**).
[14] What is chemotherapy: types of chemotherapy. May 30, 2006. www.chemocare.com/whatis/types_of_chemotherapy.asp
[15] Kostova, I. Platinum complexes as anticancer agents. Recent Pat Anti-Can Drug Discov 2006; 1: 1-22.
[16] Boulikas, T.: US2003185879A1 (**2003**).
[17] Hassan, E.M.: US2005129777A1 (**2005**).
[18] Sabel, B.A., Kreuter, J., Gelperina, S., Schroeder, U.: WO0074658 A1 (**2000**).
[19] Disis, M. Tumor immunology. June 6, 2006. www.meds.com/immunotherapy/tumor_immunology.html
[20] Roitt I, Brostoff J, Male D. Immunology. London, Harcourt Publishers Ltd. 2001.
*[21] Kuroda, S., Tanizawa, K., Kondo, A., Ueda, M., Senoo, S., Iwabuki, H.: JP2003286199A (**2003**).
[22] Kuroda, S., Tanizawa, K., Kondo, A., Ueda, M., Senoo, S., Okahima, S.: JP2004002313A (**2004**).
[23] Barchi JR, J.J., Rittenhouse-olson, K., Svarovsky, S.: WO05046722A2 (**2005**).
[24] Chari RVJ. Targeted delivery of chemotherapeutics: tumor-activated prodrug therapy, Adv Drug Deliv Rev 1998; 31: 89-104.
[26] Boettger, M., Zaitsev, S., Cartier R., Haberland, A., Sukhorukov, G., Moehwald, H., Zastrow, H., Schneider, M.: WO03087384A1 (**2003**).

[25] Bednarski, M.D., Guccione, S., Li, K.C.: US2003013674A1 (**2003**).
[27] Tian, L.R., Wei, Y.: WO04067029A1 (**2004**).
[28] Wu, K., Shi, R., Wu, D.: CN1647821A (**2005**).
[29] Sui, Y., Ye, Q., Wu, D.: CN1528452A (**2004**).
[30] Guo, T.: WO06037081A2 (**2006**).
[31] von Sonntag C. The chemical basis of radiation biology. London, Taylor and Francis 1987.
[32] Walicka MA, Ding Y, Adelstein SJ, Kassis AI. Toxicity of DNA-incorporated iodine-125: Quantifying the direct and indirect effects, Radiat Res 2000; 154: 326-30.
[33] Charlton DE, Booz, J. A Monte-Carlo Treatment of the Decay of ^{125}I, Radiat Res 1981; 87: 10-23.
*[34] Hainfeld, J.F., Slatkin, D.N.: US2005256360A1 (**2005**).
[35] Kim, C.-O., Kim, J.-H., Huang, Y.-Q., Park, S.-I.: US2005019257A1 (**2005**).
[36] Esenaliev, R.O.: US20006165440A (**2000**).
[37] Kotov, N.A.: US2005013775A1 (**2005**).
*[38] Handy, E.S., Ivkov, R., Ellis-Busby, D., Foreman, A., Braunhut, S.J., Gwost, D.U., Ardman, B., Jahngen, E.G.E.: WO03022360A2 (**2003**).
[39] Kanzius, J., Steinbrink, W.H., Hainfeld, J.F., Slatkin, D.N.: US2005256360A1 (**2005**).
[40] Chen, J.: US2002127224A1 (**2002**).
[41] Moghimi SM, Hunter AC, Morray JC. Long-circulating and target-specific nanoparticles: theory to practice. Pharmacol Rev 2001; 53: 283-318.
[42] Shefer, A., Shefer, S.: US2004234597A1 (**2004**).
[43] Dharap SS, Wang Y, Chandna P, *et al*. Tumor-specific targeting of an anticancer drug delivery system by LHRH peptide. Proc Natl Acad Sci USA 2005; 102: 12962-67.
[45] Carpenter, EE. Synthesis of magnetic nanoparticles using reverse micelles. *Nanoscience Seminar*. Copenhagen, Denmark (**2003**).
[44] Prasad, P.N., Bergey, E.J., Levy, L., Liebow, C.: WO0137721A2 (**2001**).
*[46] Sung, H.-W., Hsu, H.-K., Tu, H.: US2006115537A1 (**2006**).
[47] Prokop, A., Davidson, J.M., Carlesso, G., Roberts, D.: US2005008572A1 (**2005**).
*[48] Russell-Jones, G.J., McEwan, J.F.: EP1206251A1 (**2002**).
[49] West, J.L., Halas, N.J., Hirsch, L.R.: US2002103517A1 (**2002**).
[50] Kislev, H.: WO06051542A1 (**2006**).
[51] Juestel, T. Feldmann, C.: WO05058360A2 (**2005**).
[52] Patolsky, F., Gill, R., Weizmann, Y., Willner, I., Banin, U., Mokari, T.: WO05010211A1 (**2005**).
[53] Reszka, R., Berger, G., Pohlen, U., Jung, M.: US20016207133B1 (**2001**).