Recent progress in nanotechnology for cancer therapy

Mu-Fei Tang1,†, Lei Lei1,†, Sheng-Rong Guo1, Wen-Lin Huang2,3

1 School of Pharmacy, Shanghai Jiao Tong University, Shanghai 200240, P. R. China; 2 State Key Laboratory of Oncology in South China, Guangzhou, Guangdong 510060, P. R. China; 3 Research Department, Sun Yat-sen University Cancer Center, Guangzhou, Guangdong 510060, P. R. China

[Abstract] The application of nanotechnology significantly benefits clinical practice in cancer diagnosis, treatment, and management. Especially, nanotechnology offers a promise for the targeted delivery of drugs, genes, and proteins to tumor tissues and therefore alleviating the toxicity of anticancer agents in healthy tissues. This article reviews current nanotechnology platforms for anticancer drug delivery, including polymeric nanoparticles, liposomes, dendrimers, nanoshells, carbon nanotubes, superparamagnetic nanoparticles, and nucleic acid-based nanoparticles [DNA, RNA interference (RNAi), and antisense oligonucleotide (ASO)] as well as nanotechnologies for combination therapeutic strategies, for example, nanotechnologies combined with multidrug-resistance modulator, ultrasound, hyperthermia, or photodynamic therapy. This review raises awareness of the advantages and challenges for the application of these therapeutic nanotechnologies, in light of some recent advances in nanotechnologic drug delivery and cancer therapy.

Key words: Nanotechnology, neoplasms, therapeutics, drug therapy, combination, nanoparticles

Introduction

In recent years, significant efforts have been devoted to develop nanotechnology to enhance the delivery of anticancer drug to tumor tissue while minimizing its distribution and toxicity in healthy tissue. Many developed innovative nanotechnology platforms, such as polymeric nanoparticles, liposomes, dendrimers, nanoshells, carbon nanotubes, superparamagnetic nanoparticles, and nucleic acid-based nanoparticles [DNA, RNA interference (RNAi), and antisense oligonucleotide (ASO)], have been applied to the delivery of specific anticancer drugs, including small molecular weight drugs and macromolecules (proteins, peptides or genes). The physicochemical characteristics of nanotechnology platforms, such as composition, particle size, surface charge, surface functionalization with hydrophilic polymers, and inclusion of tissue-recognition ligands, will conduct their biodistribution and pharmacokinetics[1]. Hereby, the nanotechnology platforms could serve as customizable, targeted drug delivery vehicles capable of carrying large dose of therapeutic agents into malignant cells while avoiding healthy cells. This article overviewed current nanotechnologies for cancer therapy, focusing on the wide variety of nanotechnological platforms for anticancer drug delivery and nanotechnologies for combination therapeutic strategies.

Nanotechnology platforms for cancer therapy

The most common examples of nanotechnology platforms for cancer therapy include polymeric nanoparticles, liposomes, dendrimers, nanoshells, carbon nanotubes, and superparamagnetic nanoparticles. With small size and various structural and physicochemical features, these nanotechnology platforms can enter tumor vasculature through enhanced permeability and retention effect (EPR). The use of cancer-specific targeting residues (e.g. antibodies, ligands, and lectins) can also achieve tumor cell targeting.

Polymeric nanoparticles

Polymeric nanoparticles are prepared from natural or
synthesized polymers. Various biodegradable or unbiodegradable polymers can be used to prepare nanoparticles in order to achieve expected drug delivery performance and therapeutic effect. Among these, biodegradable polymeric nanoparticles for anticancer drug delivery have attracted great interest in recent years since they could provide controlled, sustained and targeted delivery. Polymeric nanoparticles, the most effective nanotechnology platforms, have emerged as a versatile carrier system for targeted delivery of anticancer drugs\textsuperscript{[2,3]}. Bernardi et al.\textsuperscript{[4]} investigated the effect of indomethacin-loaded nanoparticles on a xenograft glioma model in rats. The rats presented a significant reduction in tumor size and half of them presented just residual tumor cells; moreover, the animal survival rate was much larger in the drug-loaded nanoparticle group than in the control (untreated), indomethacin- and drug-unloaded nanoparticle groups\textsuperscript{[4]}.

Polymeric nanoparticles can deliver not only small molecular weight drugs but also macromolecules such as genes and proteins. A system made up of poly (D, L-lactide-co-glycolide) nanoparticles, a potent protease inhibitor (cystatin) and cytokeratin-specific monoclonal antibody, has been reported. It can neutralize the activity of excessive proteolysis in order to prevent the metastatic and invasive potential of breast tumor cells\textsuperscript{[5]}. To stabilize the surface of nanoparticle or achieve active targeting, conjugating, grafting and adsorbing hydrophilic polymers, such as polyethylene glycol (PEG), are usually used. Copolymer pegylation and folate conjugation can improve the stability of self-assemblies in aqueous medium and the tumor site selectivity in vivo of ring-opening metathesis polymerization-based copolymers\textsuperscript{[5]}. By covalent coupling of humanized monoclonal antibodies (anti-HER2) to paclitaxel-loaded poly (D, L-lactic acid) nanoparticles, immunonanoparticles were prepared to actively target tumor cells which overexpress HER2 receptors\textsuperscript{[6]}. Recently, Patil et al.\textsuperscript{[7]} produced PLA-PEG-ligand conjugate nanoparticles by a single-step surface functionalizing technique, and found that simultaneous functionalization with biotin and folic acid induced great efficacy of paclitaxel-loaded nanoparticles in a MCF-7 tumor xenograft model by enhancing drug accumulation in tumors.

Mitoxantrone-loaded polybutylcyanacylate nanoparticles (DHAD-PBCA-NPs) have presented a good effect on orthotopically transplanted hepatocellular carcinoma (HCC) in nude mice. Therefore, the activity and toxicity of DHAD-PBCA-NPs in individuals with unresected HCC were evaluated in a phase II clinical trial\textsuperscript{[8]}. The median survival was much longer in the DHAD-PBCA-NPs group than in the DHAD injection group (5.46 months vs. 3.23 months)\textsuperscript{[8]}. Polymeric nanoparticles are currently the most widely investigated nanotechnology platform for cancer therapy, despite many challenging defects or drawbacks need to be resolved before clinical application. It is also considered as the most promising vehicle for site-targeting anti-cancer drug delivery and disease diagnosis because of its good variability of chemical structures through chemical modification and the resulting flexibility of physicochemical characteristics enabling its diverse drug delivery applications.

**Liposomes**

As closed spherical vesicles, liposomes consist of a lipid bilayer which encapsulates an aqueous phase to store drugs\textsuperscript{[9]}. With the size (90–150 nm) which is slightly bigger than the conventional definition (≤ 100 nm), liposomes do not constitute novel nanotechnology, but a large portion of them are associated with nanotechnology research\textsuperscript{[10]}. Forming lipid bilayers through hydrophobic interaction, liposomes are considered as excellent platforms for the delivery of hydrophobic and hydrophilic drugs. In particular, liposomes present considerable persistence in the blood. It facilitates efficient drug delivery to target tissues. Different lipids have different fatty acid chain lengths, different head groups, and different melting temperatures. Consequently, temperature-sensitive immunoliposomes (ILs) can be constructed by manipulating the formulation. The effectiveness of 1-methylxanthine (1-MTX) as a radiosensitizer and the in vivo efficacy of the temperature-sensitive liposomal 1-methylxanthine (tsl-MTX) which combined with regional hyperthermia and ionizing radiation were evaluated\textsuperscript{[11]}. Intraperitoneal injection of the tsl-MTX inhibited tumor growth in the mouse xenograft tumor model; moreover, the combination of tsl-MTX with regional hyperthermia and ionizing radiation obviously inhibited tumor growth\textsuperscript{[11]}. Most recently, to target leukemic cells, pH-sensitive immunoliposomes (ILs) including a terminally alkylated N-isopropylacrylamide (NIPAM) in the bilayer were coupled with the anti-CDDP monoclonal antibody\textsuperscript{[12]}. The pH-sensitive ILs-CDDP immunoliposomes exhibited high cytotoxicity against HL60 cells, suggesting that the pH-sensitive immunoliposomes could be profitable in acute myeloid leukemia therapy.

Commercial liposomes have already gained approval from US Food and Drug Administration (FDA). The typical example is doxorubicin-encapsulated liposomes (Doxil), which has strong antitumor activity against a wide range of cancers.

**Dendrimers**

As highly branched artificial macromolecules with tree-like structures, dendrimers are monodisperse, three-dimensional molecules which have defined molecular weights and hostguest entrapment properties\textsuperscript{[13]}. With the
size ranging from 1 to 10 nm, dendrimers with different chemical structures and functional groups can be synthesized. Through a series of repeating chemical synthesis on the core, the size and shape of dendrimers are determined by the generation. The key useful character of dendrimers is the branches which can provide vast amounts of surface area for drugs and targeting molecules. Meanwhile, the surface functionalities, interior branching, and chemical composition of the core play a significant role in reactiviting the macromolecule.

Dendrimer is one of the most elegant nanotechnology platforms for targeted drug delivery. Conjugated with biotin as the targeting moiety, the in vitro targeting ability of partially acetylated generation 5 polyamidoamine (PAMAM) dendrimer (Ac-G5) in HeLa cells was assessed. The multi-functional conjugate Ac-G5-biotin-FITC (fluoresceinisothiocyanate) showed much higher cellular uptake than the conjugate without biotin. The energy-dependent uptake process can be blocked effectively by biotin-polymer conjugates, exhibiting an expected dose-response curve.

Nanoshells

As the layer-by-layer assembly of nanoparticles, polymeric nanoshells (20–60 nm) of diblock copolymers can be made by self-assembly of oppositely charged polymers forming a core/shell structure. With a biodegradable polymer core and mixed lipid monolayer shell, a system of folic acid-conjugated nanoparticles was developed for targeted delivery of docetaxel. Gold nanoshells (10 to 300 nm) are optically tunable nanoparticles comprising a dielectric core with a thin gold shell surrounded. In order to achieving maximal penetration of light through tissue over the near-infrared, gold nanoshells can be designed by adjusting the core radius and the shell thickness. Laser-activated gold nanoshells thermal ablation is a selective and effective technique for the ablation of prostate cancer in an ectopic tumor model.

Carbon nanotubes

As a distinct molecular form of carbon atoms which bond with each other via sp² bonds and present a hexagonal arrangement, carbon nanotubes were first discovered in the late 1980s. Conceptually, carbon nanotubes are described as well-ordered, hollow nanotubes formed when single or multiple graphene sheets are rolled into a cylinder. The two forms of carbon nanotubes are single- and multi-walled carbon nanotubes. In the family of nanotechnology platforms, carbon nanotubes have been identified as a novel tool for anticancer drug delivery. Apart from that, carbon nanotubes can immobilize molecules, such as antibodies, DNA and drugs, in order to penetrate cell membranes. Heister et al. have used an oxidized single-walled carbon nanotube, consisting of a fluorescent marker and a monoclonal antibody at non-competing binding sites, to delivery anticancer drug doxorubicin. However, because of the needle-like fiber shape, the safety of carbon nanotubes is concerned. Recently, the biological impacts (cytotoxicity, DNA damage, and inflammation) induced by different-sized multi-walled and single-walled carbon nanotubes, have been studied. The results demonstrate that long and thick multi-walled carbon nanotubes probably induce severe biological effects and may cause the augmentation of cancer risk.

Superparamagnetic nanoparticles

Superparamagnetic nanoparticles, iron oxide magnetic nanoparticles with particle sizes of about 20 nm, are composed of Fe₂O₃ or Fe₃O₄ and do not keep any magnetism after removal of the magnetic field, hence, may be used in vivo. Superparamagnetic nanoparticles can be used as contrast agents for magnetic resonance imaging (MRI), can be used for cancer thermal therapy, and can concentrate in target sites through an external magnetic field. Functionalized with recombinant single chain Fv antibody fragments (scFv), superparamagnetic iron oxide nanoparticles (SPIONs) could be used to target and image cancer cells. Conjugated to luteinizing hormone releasing hormone (LHRH), SPIONs not only achieve breast cancer cell targeting but also play the role as contrast agents in the MRI of breast cancer xenografts. The post-mortem neuropathologic studies of glioblastoma multiforme (GBM) patients treated with thermotherapy using magnetic nanoparticles were reported. Magnetic nanoparticles were injected into the tumor and then heated in an alternating magnetic field. The instillation of magnetic nanoparticles in GBM patients induced the uptake of nanoparticles in macrophages to a major extent, and the uptake was further promoted by magnetic fluid hyperthermia (MFH) therapy.

Nucleic acid-based nanoparticles (DNA, RNAi, and ASO)

Gene therapy refers to the direct transfer and expression of DNA into diseased cells for the therapeutic applications. Veiseh et al. have developed a ligand-mediated nanovector by binding the chlorotoxin (CTX) peptide and pegylation of DNA-complexing polyethylenimine (PEI) in nanoparticles which functionalized with an Alexa Fluor 647 near infrared fluorophore. Mixed nanoparticles, prepared with generations 4 and 5 poly (amidoamine) (PAMAM) dendrimers and plasmid DNA, were confirmed to be effective both in vitro and in vivo gene delivery to colon and liver cancer cells. Based on oligonucleotides, RNAi and ASO therapies can shut down the expression of...
Nanotechnology for combination therapeutic strategies

The resistance to chemotherapy is a substantial clinical problem limiting the effectiveness of anticancer drug treatment. Because of microenvironmental selection pressures, tumor cells can generate multi-drug resistance (MDR). MDR of cancer cells refers to resistance against functionally and structurally unrelated drugs. The mechanisms of MDR include sequestration, increased drug efflux, decreased drug influx, binding site modification, activation of detoxifying enzymes, blocked apoptotic signaling, and DNA repair. In order to overcome MDR, the nanotechnology for combination therapeutics has attracted increasing attention in these years. Drug delivery combined with various modulations (e.g., modulation of drug efflux, apoptotic threshold, and intracellular pH) and energy therapies (e.g., ultrasound, hyperthermia, and photodynamic therapy) have shown significant promise in enhancing MDR cancer therapy.

Drug delivery and modulation against MDR

Combining chemotherapeutic agent with MDR modulator is a common solution for overcoming MDR in cancer therapy. Overexpression of ATP-binding cassette (ABC) transporters which are involved in drug efflux, is a key factor in cancer MDR. Among various ABC transporters, P-glycoprotein (P-gp/MDR1/ABCB1) is commonly expressed in drug-resistant cells. Tarquidar, one of the third generation P-gp inhibitors, has shown marked effectiveness in early clinical trials. Patil et al. have investigated overcoming MDR by targeted delivery of paclitaxel, using tarquidar as the P-gp modulator and poly (D,L-lactide-co-glycolide) nanoparticles as the platform which were functionalized with biotin on the surface. The accumulation of paclitaxel in drug-resistant tumor cells resulted in enhanced therapeutic efficacy of the nanoparticles. After treatment with biotin-functionalized nanoparticles which loaded both paclitaxel and tarquidar (the paclitaxel dose was ineffective without tarquidar), the growth of drug-resistant tumor in mice was significantly inhibited. Several other ABC transporters also contribute to MDR, such as multi-drug resistance-associated protein (ABCC1/MRP-1) and breast cancer resistance protein (ABCG2/BCRP). However, non-ABC transporters were also studied to develop combination therapeutics for the modulation of drug efflux. One approach is the use of Cdc2. RLIP76, a non-ABC, stress-responsive drug transporter, conjugates glutathione-electrophile-conjugate (GS-E), which is over-expressed in various cancers. As a cell cycle check point control kinase, Cdc2 can bind to RLIP76 during mitosis and inhibit endocytosis. Liposomal Cdc2 delivery to H358 cells induced apoptosis, increased intracellular accumulation of doxorubicin, and decreased drug efflux from the cells. The marked obstructing effect of liposomal Cdc2 delivery on drug efflux indicates a big potential for a novel MDR cancer therapy by combining chemotherapeutic agent with Cdc2.

For MDR cancers, a high apoptotic threshold can be caused by increased expression of anti-apoptotic factors or inhibition of pro-apoptotic factors. Survivin, a member of inhibitor of apoptosis protein (IAP) family, is overexpressed in MDR cancer cells. Co-delivery of docetaxel and iSur-pDNA, a suppressor of survivin, showed combined effect of anticancer drug and gene therapy. A folate-modified multifunctional nanoassembly (FNA) was manufactured and used for the research of human HCC. Compared with free docetaxel and FNAs loading only iSurpDNA, FNAs loading both docetaxel and iSur-pDNA presented much higher cytotoxicity to HCC cells both in vitro and in vivo. The main challenge of gene therapy is the effective delivery. Combining targeted delivery of anticancer drug with the modulation of apoptotic threshold, FNAs is an efficient strategy for overcoming MDR.

As one of the distinguishing characteristics of MDR cells, decreased intracellular pH can be exploited to design nanoparticulate delivery. In order to improve the therapeutic efficacy of doxorubicin (DOX) on MDR cancers, two polymeric nano-conjugates were developed and micelles based on the conjugates were decorated with avP3 integrin-targeting ligand (RGD4C) for active cancer targeting. Using the pH-sensitive hydrazone bonds, DOX was conjugated to the degradable poly (ethylene oxide)-block-poly (3-caprolactone) (PEO-b-PCL) core in the first formulation, which named RGD4C-PEO-b-P (CL-Hyd-DOX); the second formulation, named RGD4C-PEO-b-P (CL-Ami-DOX), was obtained by conjugating DOX to the core using the more stable amide bonds. Based on AFM and DLS, both two block copolymers-DOX conjugates self-assembled into spherical nanoparticles (60 to 90 nm). Both micelles did not release detectable DOX levels at physiological pH of 7.2, but the release significantly increased at pH 5.0. In vitro studies showed that both micelles decorated with RGD4C can effectively increase the therapeutic efficacy of DOX on human MDA-435/LCC6 MDR cancer. The active targeting of pH-sensitive carriers may further decrease the cytotoxicity to normal cells, thereby conferring a benefit on the treatment of aggressive MDR cancers.

Drug delivery and energy therapies

Ultrasound is used in many medical applications,
especially cancer therapy. Recently, the ability of ultrasound to induce controlled release of tumor-localized drugs from nanotechnology platforms has been studied. Schroeder et al. exposed Stealth cisplatin to low frequency ultrasound (LFUS) (3.3 W/cm²) for different periods of time (30–180 s) to induce a time-dependent release, and achieved a release amount of 62% after 180 s of LFUS irradiation. Then, the ability of LFUS to improve the anticancer efficacy of cisplatin-loaded nano sterically stabilized liposomes (nSSL) was studied. In J6456 murine lymphoma tumors exposed to LFUS, about 70% of cisplatin was release, which was much larger than those not exposed to LFUS (<3%) [49]. The therapeutic efficacy of LFUS-induced localized cisplatin release was studied on BALB/c mice bearing C26 colon adenocarcinoma [50]. Compared with other groups, the group treated by cisplatin-loaded nSSL combined with LFUS had the best therapeutic effect that tumors not only stopped proliferating but also regressed over time [50]. Using ultrasound to enhance the drug release of targeting carriers is probably a viable technique for MDR cancer therapy.

Many studies on overcoming MDR also focus on combing drug delivery with hyperthermia. Combining hyperthermia with folate receptor-targeted thermosensitive magnetic liposomes loaded with doxorubicin (MagFolDox) was described [49]. At 42.5°C and 43.5°C, magnetic hyperthermia significantly increased the tumor cytotoxicity of MagFolDox [50]. It indicates that a system of physical and biological drug-targeted delivery combined with hyperthermia can be used advantageously for cancer therapy.

Photodynamic therapy (PDT) is a therapeutic modality used in cancer treatment. Combination of drug delivery and PDT has been used to target MDR cancer cells. Khadir et al. have studied the effect of doxorubicin nanoparticles in combination with PDT on MDR cancer in a mouse tumor model. Surfactant-polymer hybrid nanoparticles, which composed of Aerosol-OT™ (AOT) and sodium alginate, were used for synchronized delivery of doxorubicin and methylene blue [51]. With Balb/c mice bearing mammary adenocarcinoma as a MDR tumor model, the combination therapy significantly enhanced drug accumulation and inhibited tumor proliferation. Combining PDT with drug-loaded nanoparticles may be a promising technique to overcome MDR in cancer therapy.

Advantages and challenges of nanotechnology for cancer therapy

Nanotechnology has many advantages in cancer therapy. With small size, nanotechnology platforms can enter tumor vasculature via EPR. Besides, functionalization with hydrophilic polymer/oligomer can offer a long circulation half-life and prolong the exposure time of tumor tissue to anticancer agents; whereas inclusion of tissue-recognition residues, such as antibodies, lectins and ligands which are specific for cancer cells, can help nanotechnology platforms achieve tumor cell targeting. For overcoming MDR of cancer cells, a major challenge in effective cancer therapy, combinations of multi-functional nanotechnology platforms and other therapies have been developed and achieved significant successes. However, there are still challenges to the development and application of nanotechnology platforms in cancer therapy, such as limited knowledge of the cancer cell physiology, small variety and poor functionalization of medical nanomaterials, and deficiency of clinical evaluation criteria. Nonetheless, with further advances in functionalization base on thorough understanding of the physiological features of cancer cells, nanotechnology platforms hold the promise of essentially changing the practice of oncology, allowing easy and effective targeted therapies.

References