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REVIEW ARTICLE

Trends of Gold Nanoparticle-based Drug Delivery System in Cancer Therapy

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Following surgical removal of malignant tumors, chemotherapeutic intervention usually is subsequently applied in patients with advanced stages of cancer. Most chemotherapeutic drugs are intravenously injected into patients, leading to systemic cytotoxicity in organs and tissues, including healthy tissue and tumors. Currently, it has been demonstrated that gold nanoparticles can easily penetrate blood vessels and tissue barriers into tumor foci, which indicates gold nanoparticles as a more effective drug carrier with great merits in reducing cytotoxicity and economic burden in patients. Moreover, gold nanoparticles display several unique characterizations with multiple functions in therapeutics, imaging, and surface modification, suggesting gold nanoparticles may become effective antitumor drug carriers. In this review article, we discuss the limitations and applications of gold nanoparticles in surface modification, targeting strategy, and safety considerations.

1. Introduction

Cancer is the leading cause of death worldwide. According to the United States cancer statistic reports, one of every four deaths in the United States is caused by cancer.[1] Chemotherapeutics are commonly used in current practice to treat cancer via intravenous administration but also to elicit toxicity to normal cells, leading to severe side effects in patients. Therefore, a new and improved therapeutic method to target tumor foci coupled with enhanced cytotoxicity on cancer cells and decreased side effects is needed. Recently, inorganic nanoparticles such as gold nanoparticles (GNPs) have been explored and exploited as a promising candidate for various biotechnology applications because of their unique characterizations.

GNPs have been used as nanobiomaterials for molecular imaging and drug delivery in recent years.[2] Gold nanoparticle conjugates express unique properties such as increased binding affinity and selective targeting to specific tissue or cells when delivered systemically.[3] Because gold nanoparticles can be modified in different ways by binding specific receptors coupled with various forms of therapeutics, there is a wide range of research and nanoparticle-based therapeutic methods under development for cancer.[4,5] The delivery of GNP-conjugated drugs have a higher perfusion rate in targeting tumor foci, leading to reducing antitumor drug dosage for treatments and lower toxicity to normal tissues coupled with less side effects.

In this review, we discuss various drug delivery systems of GNPs in cancer, including targeting approaches, modified conjugates and safety issue using nanoparticles in GNP-based drug delivering system.

2. Nanotechnology and nanomedicine

Nanotechnology is continuously being extended in the field of medicine to reach maximum therapeutic possibility and reduce side effects of clinically used agents. The history of nanotechnology began in the 1950s, when the first polymer drug conjugate was successfully schemed by Jatzkewitz,[6] followed by the liposome discoveries of Bangham and Horne,[7] and Bangham et al[8] during the mid 1960s. Current nanotechnology applications in medicine led to the emergence of a new domain in science known as nanomedicine, offering some exciting prospects such as improvement in diagnosis, monitoring, prevention, and treatments of disease using selectively active drug carriers, diagnostic agents, and pharmaceutical moieties to a target site.[9] Various types of nanoparticles

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have been used in biomedical applications, including drug delivery, molecular imaging, and combined therapy and diagnosis.

In the field of medicine, nanotechnology is the basis of nanocarriers (i.e., nanoparticles, polymeric micelles, liposomes, dendrimers, and carbon nanomaterials) and drug conjugates no larger than 200 nm.[10–13] One of the key discoveries observed by Matsumura and Maeda[14] in the 1980s revealed that nanoparticles could accumulate in tumors, leading to the scientists' interest in the application of nanoparticles as antitumor carriers. In normal or healthy tissue, the vascular endothelial layer is highly deployed and arranged with a barrier function in preventing the passage of molecules. With tumor progression, neovasculatures are formed and characterized as highly disordered vascular endothelial layer with large gaps between cells, resulting in leaks and propensity for the passage of molecules. Therefore, the unique abnormal structure, loose pattern, and less integrity of the vascular endothelial layer of cancerous blood vessels contribute to greater permeability and then facilitate the increased deposition of substances/particles onto solid cancerous tissue, phenomena termed as the enhanced permeability and retention effect (EPR).[14–16] Because EPR effect increases nanoparticle accumulation in solid tumor sites by passive targeting, nanoparticles as drug carriers exhibit a significant effect on enhanced therapeutic efficacy with reduced side effects and cytotoxicity to other tissues and organs (Figure 1).[17,18]

3. Gold nanoparticles

In general, gold nanoparticles are synthesized by the chemical reduction of chloroauric acid (HAuCl4) using reducing agents. Gold nanoparticles exhibit a combined feature of chemical, physical, optical, and electronic properties and may be applied as a new platform to bring about benefits in various fields, such as medicine.[19–25] Because of the unique property of a wide range of core sizes from 1 nm to 150 nm, GNPs can be easily modified with controlled dispersal. Therefore, GNPs have a great potential to be used as a drug delivery system for efficient drug transport into different cell types (Figure 2). Because of the ease of functionality and tailoring surface of GNPs, modified GNPs gain accessibility and effectiveness to target cancerous tissues by either active or passive targeting mechanistic system.[26,27] The size of GNPs determine the optical property in UV absorbance, and the color from red or blue.[28] The GNP surface is one of the most stable and easily functionalized platforms for further modifications such as adding substance or molecules forming a specific monolayer to prolong stability and enhance dispersion in organic media, and for further conjugations of targeting probes or drugs.[29] Gold nanoparticles have brought about a new direction and theological ideas to build better and more effective diagnostic and therapeutic agents for different biomedical-based applications in the current biotechnology industry. Currently, two of the nano-based products are under investigating in clinical trials with United States Foot and Drug Administration approval (Table 1).

4. Targeting approaches

It has been well documented that the passage or unloading of antitumor drugs onto targeted tumor sites relied on several permeating mechanisms including passive targeting, active targeting, or a combination. Active targeting uses GNPs that are preconjugated with various probes or targeting agents including antibodies, small molecules, or peptides to locate and attack tumors. Passive targeting is simply taking advantage of the EPR effect to deposit antitumor drugs to tumors (Figure 3), which is a common characteristic of nanoparticle-based drug delivery to cancer.[30] To enhance antitumor drug targeting to tumor coupled with better therapeutic efficacy, most nanoparticle-based carriers are theoretically labeled with tumor targeting probes. Previous studies suggests that the optimal size of nanocarriers for attacking tumors

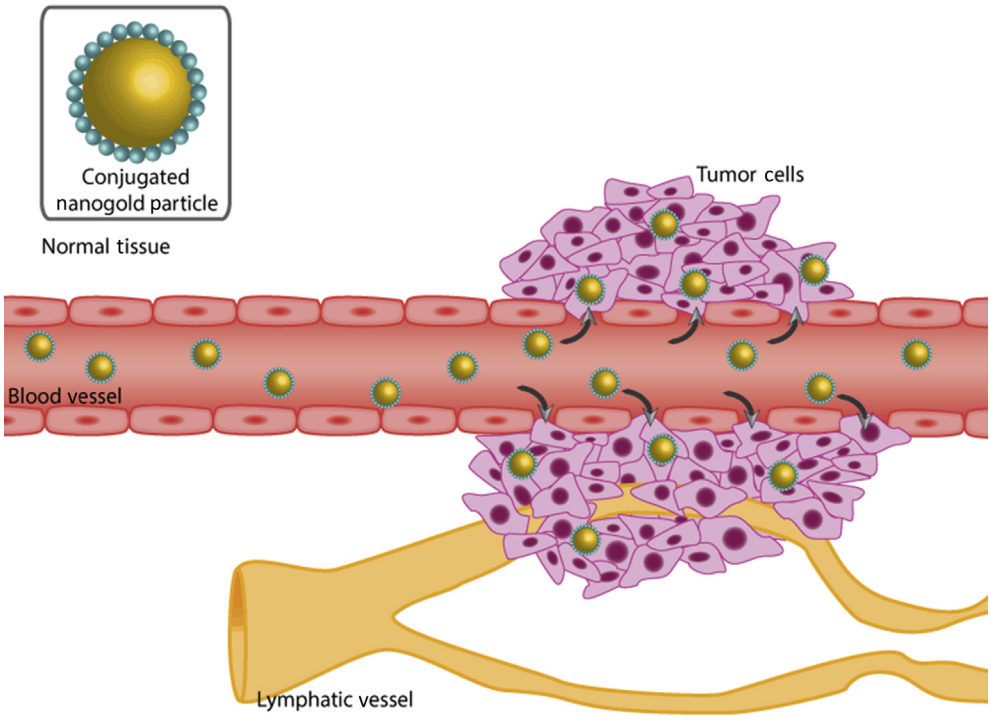

Figure 1 Graphic illustrating the accumulation of circulating gold nanoparticle conjugates at tumor sites by the enhanced permeability and retention effect. Because of disordered endothelial cells, gold nanoparticles can penetrate through blood vessels at the tumor site. The tumor site has diminished lymphatic vessels that reduce the gold nanoparticle clearance from the tumor.

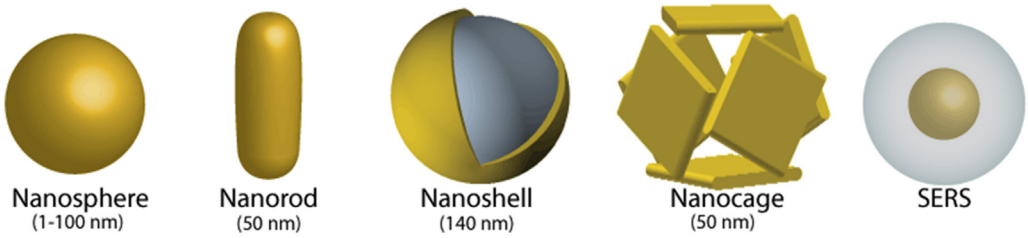

Figure 2 Different types of gold nanoparticles commonly used in anticancer diagnosis and therapeutic applications are shown.

should range from 10 nm to 200 nm and premodified with caution charges on the nanocarrier's surface. Without positively- or negatively-charged modifications on the shell surface, those nanoscale carriers would be rapidly cleared by the reticuloendothelial system.[31] Thus, the most common surface coating agent for protecting and reducing reticuloendothelial system-mediated clearance is to decorate nanocarrier surface with polyethylene glycol (PEG).[32,33] For instance, the targeting efficacy of tamoxifen carried by GNPs with probes of single-chain variable fragment (ScFv) antibody to tumors was significantly increased when it was precoated by thiol-PEG, highlighting the importance of the charge modifications on GNP surface.[20,28] In addition to the passage of vasculature, drug carriers must be internalized to cancer cells and achieve cytotoxic effects via endocytosis or other mechanisms. Without successful internalization into cells, therapeutic efficacy will be low. Previous studies indicated that GNPs sized approximately 50 nm have a higher rate for cancer cell internalization.[34] However, another study demonstrated that the ultra-small (2.7 nm) GNPs conjugated with doxorubicin are successful in mediating apoptosis in the resistant cancer cells, suggesting ultra-small size GNPs may also be lethal to normal cells. These studies suggest that the size of GNPs is a crucial parameter for passage of the vessel wall, cell internalization, and size-mediated cytotoxicity to normal cells. Based on this information, we may develop a rational size or sizes of GNPs for various types of cancers depending on the characteristics of vasculature and the form of cancer. Both parameters including the size of drug nanocarriers and surface charges are important in designing chemotherapeutic GNPs.

Furthermore, in addition to the size and surface chemistry of GNP, the shape of nanoparticles is an important parameter for cellular uptake. For instance, spherical citrate GNPs with 74 nm and 14 nm core diameters showed greater uptake by cells. Furthermore, shorter gold nanorods displayed higher rates in uptake by cells than do the longer nanorods.[34] In general, the uptake capacity of spherical forms of GNPs by tumor cells is much greater than that of the rod-shaped GNPs.[35]

Table 1 Current cases using gold nanoparticle-based therapeutics to cancers under investigation with Food and Drug Administration (FDA) approval

Name	Ingredient	Target	Current status
Aurimmune	PEG-Thiol Gold nanoparticles modified with TNF-α (~27 nm)	Solid tumor	Phase II
AuroShell	Silica nanoparticles coated with Gold (~150 nm)	Solid tumor	Phase I
Verigene (*In-vitro* diagnostic purpose)	Gold	Genetics	FDA approved

PEG = polyethylene glycol; TNF = tumor necrosis factor.

Furthermore, a lower pH value in the cytosol part of tumor cells has been characterized and may facilitate drug release after internalization of carriers through a passive targeting mechanism. Because of the relatively acidic condition of the cytosol of cancer cells, formulated pH-sensitive GNP-based drug carriers have been introduced and may lead to accelerated acid-sensitive antitumor drug release into the cytoplasm, resulting in an increased concentration of drugs in the cytosol with an enhanced cytotoxic effect on the targeted cells.[36]

In active targeting, gold nanoparticles can be conjugated with specific targeting moiety to permit preferential accumulation within cancer cells. Several modifications including carbohydrate moiety, specific antibodies, ligands, and antigenic agents have been applied to archive and reinforce active targeting through interactions between counterpart receptors expressed on cells

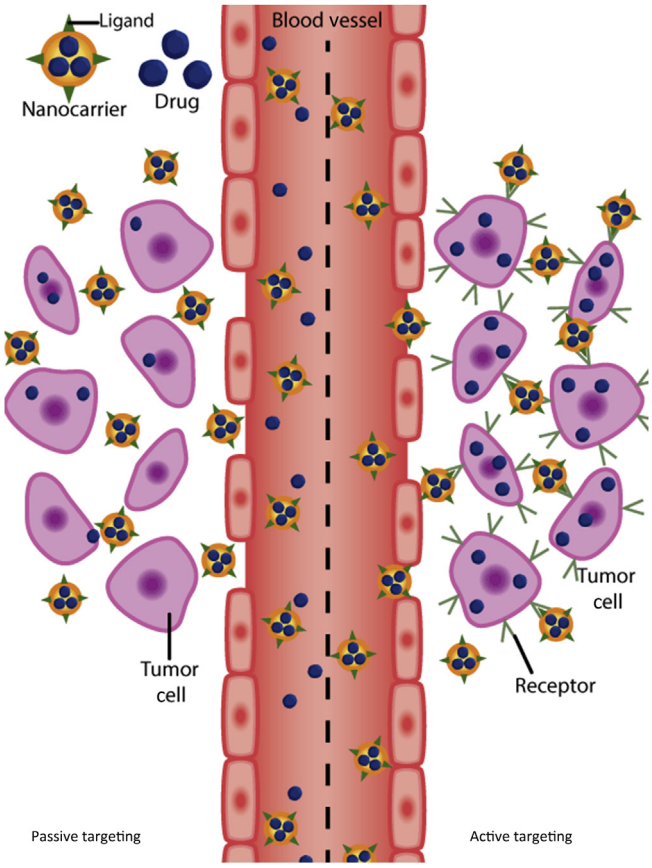

Figure 3 Graphic illustrating the accumulation of gold nanoparticles delivering drug into the tumor sites by passive or active targeting. Gold nanoparticle carriers reach the tumor site selectively through the leaky vasculature in tumor. After nanocarriers penetrate the tumor, targeted nanocarriers can bind or enter the cell via receptor-mediated endocytosis.

followed by initiation of endocytosis[37] (Figure 3). Many types of cancer cells overexpress folic acid receptors on their surfaces that are good candidate markers for GNP-based drug active targeting driven by the decoration of acid and methotrexate derivatives on particles. In addition to therapeutic application, GNP-based carriers are also used in imaging for diagnosis or monitoring in cancer progression after treatment. Therefore, GNPs can be exploited to link to target imaging molecules combined with therapeutic compounds for imaging cancerous tissues.[38–40] Previous studies indicated that conjugation of folic acid to PEG-coated GNPs could directly target folate receptor-positive cells.[41,42] Furthermore, another case using methotrexate successfully inhibited tumor growth of lung carcinoma.[43] This study suggests that methotrexate and folate are putative candidates for assisting tumor targeting.

In addition to the alteration in morphological and biological characteristics of tumor microcirculations in comparison with normal vessels, the cancer cells also display the differential markers on their cell surface. For example, several types of tumors are expressed with numerous and/or various surface receptors such as hormone receptors, which may be associated with the progression and/or malignancies of breast cancers and lung cancers. According to clinical statistics, most breast cancers express increased estrogen receptors, whereas higher levels of androgen receptors are expressed on prostate cancers.[44,45] Based on these observations, anti-estrogen-bound GNPs such as tamoxifen were evaluated *in vitro* for the treatment of breast cancers. The results show the therapeutic GNPs are selectively delivered to estrogen receptor-positive MCF-7 breast cancer cells in a ligand-receptor mediating manner.[3,46] Combinations of two selective agents or molecules to synergistically target tumors are also characterized. The results of previous studies suggested that therapeutic GNPs with dual receptor-targeting probes including folate acid and epidermal growth factor shows higher efficiency in transporting and targeting ovarian cancer cells, which typically overexpress folate and epidermal growth factor receptors.[47] This study suggests that the frequency of dual ligand-labeling GNPs targeting ovarian cancer cells may be greatly enhanced.[48]

Neuropilin-1 receptor, a transmembrane glycoprotein and mediating in angiogenesis and vascular permeability, is another candidate for drug targeting. It has been well characterized that many types of tumors including osteosarcoma, lung cancer, brain tumors, pancreatic tumor, and others express an increased level of neuropilin-1 receptors.[49,50] A recent study indicates that glutathione-coated GNPs conjugating with platinum at 5.2 nm bind to neuropilin-1 receptors at the oligosequence of Cys-Arg-Gly-Asp-Lys (CRGDK).[51]

Targeting therapy to hepatocytes is also very important for improving the prognosis of liver cancers. The work conducted by Garg et al[52] reveals that glutathione concentration is an important factor in hepatocyte targeting using the lactose surface-modified gold nanoparticles with fluorescence-reporting drugs. Results demonstrated that drug release from therapeutic GNPs into cytosol is selectively efficient only in those hepatic cells expressing high glutathione concentration. Therefore, it suggests that tumor-targeting biomolecules and the target-specific ligands can carry and unload drugs to certain types of tissues in a more efficient fashion. Previous studies demonstrate that tumor-targeting molecules and size of GNPs may affect depositions of therapeutic GNPs within cancer cells. Furthermore, previous studies reveal that compound-modified GNPs gain more resistance to metabolic clearance in the bloodstream. PEGlyated GNPs grafted with galactose (Gal-PEG-GNPs) increase stability and half-life in blood circulation, leading to comparatively higher targeting into the liver.[53] Certain studies, however, showed no significance differences in tumor targeting and drug accumulation either in passive- or active-mediating targeting, suggesting that GNPs can reach tumor sites simply via the EPR effect.[32,54]

5. Efficacy of GNP conjugates

Several factors such as solubility, stability, and nonspecific biodistribution determine the *in vivo* affinity to targeting the specific cells. To optimize therapeutic GNPs, two important issues need to be considered, including (1) the reduced the general cytotoxicity in normal cells with lower side effects and (2) the surface modification of GNPs prelabeled either with biomolecules or therapeutic agents. In addition to molecules such as active ligands, antibodies, and other molecules to facilitate drug delivery to target various issues as previously described, oligonucleotide, peptide/protein, carbohydrates, and lipids can also enhance the rate of endosomal escape, contributing to increased therapeutic efficacy.[55] In addition to PEG and other organic agents, RNA or DNA are also used by packing into the spherical form of nucleic acids and then wrapping around therapeutic GNPs. In addition to antibodies, peptides, and small molecules, nuclear acid is also able to conjugate to the spherical form of nucleic acids, which may implicate the great potency in diagnosis and treatments on gene-associated disease.[56,57] A previous study suggests that hairpin GNPs can enter cells by membrane-nanoparticle interactions coupled with endocytosis mechanism.[58] Because oligonucleotides possess multivalences, oligonucleotide-bound GNPs display a dual advantage in binding affinity and carrying capacity. The protein surface modification on GNPs provides better biocompatibility and less toxicity to normal cells. A recent study showed that albumin-coated GNPs at 15 nm can improve targeting to the endothelium of the brain, lungs, liver, and kidneys 30 minutes after administration.[59] Moreover, a recent contradictory study demonstrated that bovine serum albumin (BSA)-capped gold nanoparticles GNPs conjugated with MTX (BSA-GNP-MTX) exhibits more cytotoxic effects by inhibiting the cell proliferation of breast cancer cell line MCF-7 as compared to the free drug methotrexate alone without GNP carrier mediating.[60] However, it has been suggested that BSA coating on GNPs can protect from hemolysis and reduce particle-associated cytotoxicity.[61] Thus, BSA modification on the surface of GNPs is considered to be safe and useful. Antitumor drugs such as cisplatin, carboplatin, and oxaliplatin are common and very potent chemotherapeutics to several forms of cancers. Previous studies suggest that GNPs can improve therapeutic effects of platinum derivatives.[62] For instance, oxaliplatin-thiolated poly (PEG) GNPs display a higher penetration rate to the nucleus of lung cancer cells, resulting in greater cytotoxicity compared to oxaliplatin alone. Furthermore, tumor necrosis factor-α (TNF-α)-GNPs has currently been studies for therapeutic potential; ongoing results reveal the great safety and tolerance at clinical trial Phase I.[5,63] Because most tumor cells are ready to develop drug resistance after the first initial treatment of antitumor drugs, a theological therapeutic strategy needs to be considered to completely eradicate cancer cells shortly after the initial chemotherapy regimen.[64] Currently, several cytostatic drugs such as doxorubicin, cisplatin, and capecitabine have been used in various forms of cancers. Interestingly, L-aspartate-coated GNPs-cytostatic drugs displayed a longer and more profound cytotoxicity in cancer cells as compared to cells only exposed to cytostatic drugs, suggesting GNPs may contribute additional therapeutic effects.[65] Drug resistance of tumor cells is formed mainly because of the pumping out of drugs through specific efflux pumps mediated by energy-dependent drug transporters, or tumor cells evolve the insensitivity to the apoptotic program induced by antitumor drugs.[66] To overcome or prevent drug resistance during the therapeutic course, multiple functional modifications on GNPs need to be applied. GNPs are simultaneously conjugated with two

therapeutic agents, for example, doxorubicin (DOX) and antibodies against to the death-4 receptors (DR4). The DOX-DR4-GNPs reveal a greater therapeutic potential as compared to either drug alone conjugated on GNPs.[67] These results demonstrated that combined DOX (a chemophotothermal agent) and DR4 antibodies exhibit stronger cytotoxicity to tumors and less dosage is required for therapy. Therefore, GNPs-conjugated with anticancer therapeutics may provide cancer patients with better a therapeutic outcome and reduced therapeutic expense. Furthermore, this compound or cocktail of therapeutics may prevent or delay drug resistance and achieve successful treatment.

6. Safety issue in using GNP as a drug carrier

The properties of GNPs in size, shape, surface chemistry, and mechanical features determine the toxicity, stability in blood, and permeability and internalization of cells.[68–71] Because of new varieties and surface modifications of GNPs, the cytotoxicity of GNPs should be determined. Previous studies indicated that *N, N, N*-trimethylammoniumethanethiol–coated GNPs at a size of 1.3 nm potentially induced systemic cytotoxicity to the embryos of zebrafish, leading to the development of small, malpigmented eyes and neuronal damage in the developing zebrafish.[72] Furthermore, PEGylated GNP at a size of 15 nm induced hepatotoxicity in rodents with malnutrition.[73] Pathological results revealed that severe hepatic cell damage, acute inflammation, and increased apoptosis were observed in livers along with significantly elevated reactive oxygen species production. Therefore, it suggests that the patient's health should be assured and clarified prior to the administration of therapeutic GNPs.

The size of GNPs is also an important parameter for stability of GNPs conjugates in blood circulation. Previous studies indicate that 10 nm GNPs can circulate in blood for >24 hours in animals, but other sizes of GNPS remain for a shorter time.[74] In addition to stability in blood, the size of GNPs is also strongly associated with cytotoxicity and aggregation in blood cells. Trisphenylphosphine-coated GNPs at a size of 1.4 nm display the most cytotoxicity by inducing cell necrosis on tissue fibroblasts, epithelial cells, macrophages, and melanoma cells as compared to larger GNPs.[75] Moreover, the tissue specificity and the biodistribution of GNPs are decided by the cell surface properties, underlining the ratio of endocytotic and exocytotic activity among cells.[76] However, the relatively larger sizes of GNPs at diameters of 50 nm or 200 nm exhibit no cytotoxicity to animals.[77] Moreover, another contradictory study indicates that Wistar rats that inhaled 18-μg GNPs at 2 nm, 20 nm, or 200 nm showed nongenotoxic, systemic, or local adverse effect in lung.[78] Furthermore, additional modifications on the surface in GNPs may alter cytotoxicity and stability. For example, PEG coating can enhance the stability of gold nanorods in the bloodstream of mice, but not improve the cellular internalization of GNPs.[79] However, an alternative coating of glutathione can improve GNPs to target lung tissue without eliciting inflammation or causing morbidity in animals.[80] The general cytotoxic mechanism is first initiated at internalization of GNPs by endocytosis and then extensively release of relatively toxic ions, leading to a specific ion toxicity effect coupled with inactivation of associated enzymes, depolarization of mitochondria membrane, perturbations of cellular redox balance, and lysosome dysfunction in cells. These cellular injuries increased reactive oxygen species levels in cells, inducing apoptosis, and accelerating cell membrane damage. Future *in vivo* studies are needed to collect the aforementioned parameters, including aggregation of blood cells cytotoxicity caused by the accumulation of GNPs in endosomes or the endoplasm reticular system, and genotoxicity. Moreover, we also need to take special attention in possible immune responses mediated either by GNP or their surface conjugates.

Table 2 Advantages and limitations of gold nanoparticles in cancer drug delivery application

Advantages
Ease to synthesis with controlling over size and shape
Simple ligand conjugation
Optical properties depending on shape and size
Biocompatible
Multifunctional vehicles as drug delivery, imaging, and therapy
Stability
Limitations
Nonbiodegradable, nonporous
Surface modification alters biodistribution, toxicity or phamacokinetics
Lack of information on toxicity and interaction to living cells

7. Conclusion and challenges

Because of unique physical and chemical properties, GNPs have received a great deal of attention in biomedical applications. The combination of GNPs and anticancer treatment strategy presents tremendous opportunities in nanomedicine, especially for the therapeutic management of cancers leading to more therapeutic efficacy and fewer side effects. This new approach includes photothermal therapy, gene delivery, cell cycle regulation, and targeted drug delivery. GNP conjugates show multivalence and functional versatility, which can exhibit increased binding affinity, longer circulatory half time, improving biocompatibility, enhanced internalization within cancer cells, and much higher targeting selectivity of drugs to tumors. It is highly possible that GNPs could provide a bright future for biotechnology and the pharmaceutical industry for improving cancer therapy. So far, several GNPs-based drugs are undergoing clinical trials including TNF-α–conjugated GNPs for solid tumors.[5] To achieve high and efficient drug delivery to tumors, GNPs surface modifications are needed, to add guiding moieties such as ligands, antibodies, and other directing agents to enhance selectively targeting into a specific tumor. The limitations of GNP-based drug carriers include cytotoxicity, interactions with healthy cells, aggregation in the bloodstream, and nonbiodegradability (Table 2). Even though many reports and studies indicate that GNPs are relatively safe to use in clinical applications, some contradictory results suggest that we need to pay more attentions to GNPs-induced cytotoxicity. Therefore, more cytotoxicity assessments of GNPs *in vivo* should be performed.

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