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Gold nanoparticles for cancer theranostics: A brief update

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Gold nanoparticles (AuNPs) exhibit superior optical and physical properties for more effective treatment of cancer through incorporating both diagnostic and therapeutic functions into one single platform. The ability to passively accumulate on tumor cells provides AuNPs the opportunity to become an attractive contrast agent for X-ray based computed tomography (CT) imaging *in vivo*. Because of facile surface modification, various size and shape of AuNPs have been extensively functionalized and applied as active nanoprobes and drug carriers for cancer targeted theranostics. Moreover, their capabilities on producing photoacoustic (PA) signals and photothermal effects have been used to image and treat tumor progression, respectively. Herein, we review the developments of AuNPs as cancer diagnostics and chemotherapeutic drug vector, summarizing strategies for tumor targeting and their applications *in vitro* and *in vivo*.

Keywords: Gold nanoparticles; multimodal imaging; phototherapy; gene therapy; drug delivery.

1. Introduction

Despite significant advances in anti-cancer therapies, surgery in conjunction with chemoradiotherapy remains the first and gold standard for fighting against malignant cancer. Even after removing tumor tissues, many cancers are still diseases with dismal prognosis because of poor early detection and therapeutic options for their highly metastatic forms. Hence, there is an urgent need to develop novel technologies for early detection and treatment of cancer in order to increase patient survival. Meanwhile, awareness of the side effects patients endured during chemotherapy and radiotherapy, local approaches are necessary for improving the qualities of patients' lives. Therefore, designing effective targeted therapies related to cancer are needed for precise treatment and predicting the patient's prognosis.

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Recent years, nanotechnology has been a hotspot of medical research, and many nanoparticles (NPs) have been developed as detectors of cancer cells. The radioactive, optical or magnetic properties of some NPs provide effective means to image tumors at their early stages of development. Some of them can be functionalized as anti-cancer nanovehicles which permit to load drugs and deliver them directly to tumor. The rise of NPs as cancer theranostics (therapy + diagnostic) is expected to dramatically improve the cancer management.

Metal-based NPs (MNPs) are commonly designed as theranostic materials for cancer cells through functionalized ligands targeting tumor biomarkers. Most of them can accumulate and be easily detected in diseased tissues, when these MNPs are exposed to excitation sources. Nanoconstruct combined functionalized metal shell with encapsulated cancer killer as core is another method to treat local tumor precisely. MNPs, especially frequently used gold (Au) and iron oxide (IO) NPs, are expected to play promising roles in clinical practice based on their structure stability, size variability, controlled release and low toxic effects during cancer theranostics. This short review focuses on AuNPs used as theranostics of the latest oncological research. Breakthroughs in design of AuNPs for early detection and local treatment of tumors are highlighted.

2. Multimodal Imaging of Gold Nanoparticles

2.1. *AuNPs based cancer optical diagnostics*

AuNPs are available for attaching diverse biomolecules because of their large surface-to-volume ratio. They have unique optical and electronic properties due to the fact that collective oscillation of conduction electrons on the surface of monodispersed and spherical AuNPs with the sizes of $\sim$ 10 nm lead to strong surface plasma resonance (SPR) absorption and scattering intensity at around 520 nm, which show a deep red color in aqueous solution simultaneously.[1] As excellent imaging probes, the SPR of AuNPs can be readily tuned by changing sizes, shapes and compositions of NPs.[2] Increasing the diameter of AuNPs via synthesis or self-aggregation provides the color change from red to purple. Therefore, gold-based NPs are widely used to detect various biological molecules of interest and identify the accumulation of their targets *in vitro* and *in vivo*.[3]

AuNPs have been associated with an important application in surface enhanced Raman scattering (SERS) based on its SPR. Raman spectroscopy can yield a very narrow spectral vibration characteristic of the investigated sample.[4] Gold nanocores surrounded by Raman organic molecules dramatically amplify the intensity of the Raman signal via SERS. The functionalized AuNPs offer a noninvasive technique to detect early disease *in vitro* and *in vivo* due to moderate depth penetration of the optical beam and their negligible toxicity.[5,6] Affibody functionalized AuNPs for Raman molecular imaging of the cancer biomarker epidermal growth factor receptor (EGFR) have been used to successfully detect colon cancer both in cell cultures and xenografted animals.[7]

Hollow AuNPs and nanoporous Au are another two promising candidates for novel SERS substrates. They can be developed as highly active, stable, biocompatible and reusable SERS substrate.[8] The hollow and surface of AuNPs may provide two different functions for optical identification. SERS based imaging of functionalized hollow AuNPs may make a rapid, accurate and nondestructive method to distinguish different phenotypes of breast cancer from normal cells come true through simultaneously characterizing and quantifying the special biomarkers of tumor phenotypes.[9] The optical properties of various AuNPs have been exploited intensively in cancer detection applications due to their sensitive and selective response to malignant tumors.

The fluorescence quenching ability of AuNPs also can be applied to develop fluorescent molecular probes through fluorescence resonance energy transfer (FRET). The FRET-based AuNPs monitor the interactions between various biomolecules and AuNPs through detecting the fluorescence quenching in donor or signal appearance in acceptor of AuNPs.[10] New AuNPs immobilized double labeled peptide on their surface have been designed and synthesized to simultaneously sense and image the different expression levels of two enzymes which have been considered associating with tumor metastasis in living cells under 350 nm wavelength excitation. Cancer and normal cells can be easily distinguished through apparent difference in fluorescence imaging.[11]

2.2. *AuNPs-based tumor contrast imaging in vivo*

AuNPs not only can be used for cells imaging, but also can be applied to CT imaging *in vivo*. They have been treated as an attractive contrast agent for X-ray-based CT because of their higher absorption coefficient, easier attachment to targeting moieties, longer clearance time and better body tolerance than iodine agents.[12] Higher concentration and smaller size of AuNPs show greater X-ray attenuation. Thus, a certain size range of AuNPs can passively accumulate on tumors through the enhanced permeation and retention (EPR) effect and yield a distinguishable X-ray attenuation, which is not typical for normal cells or tissues. It is an easy way to diagnose cancer with highly distinct images. AuNPs have been demonstrated for hepatocellular carcinoma and breast cancer detection *in vivo* by this kind of nonselective approach.[13,14]

Conjugation of peptides, antibodies, aptamers or small molecules that possess high affinity toward specific molecular signatures found on cancer cells onto AuNPs surface can be selectively accumulated on tumor cells or tissues. Molecularly targeted AuNPs reach tumor tissues with increasing signal via binding biomarkers of cancer cells and subsequently remaining at the tumor site for extended durations. The specific targeting approach has been used to image prostate, small cell lung carcinoma, head and neck cancer, millimeter-sized human breast tumors in mice and human lung adenocarcinoma with actively targeted AuNPs as CT contrast agents.[15–18] The decorated AuNPs have been transformed cancer diagnosis based on anatomical structures into molecular imaging.[19]

Moreover, magnetic resonance (MR) imaging is another powerful noninvasive medical imaging modality which affords better resolution as CT and higher sensitivity than other imaging techniques.[20] For accurate cancer diagnosis, it is essential to develop various contrast agents for multimode imaging applications. The multifunctional AuNPs have been fabricated as dual-modality contrast agents for CT/MR imaging of breast cancer cells MCF-7 and human epithelial carcinoma cells KB xenografted tumor model *in vivo*, respectively.[21,22] After modified with the ligand of tumor-specific receptor, the multimode imaging agents can be used for accurately diagnosing different types of cancer.

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging as the other two important modalities of molecular imaging have received special attention due to using high sensitivity, translational capability and unlimited tissue penetration of radiolabeled AuNPs.[23,24] Copper-64-alloyed AuNPs have been used for cancerous PET imaging owing to their facile synthesis, radiolabel stability, diagnostic accuracy and rapid systematic clearance.[25,26] Also, AuNPs based PET/MR and PET/UV-Vis dual-modality probes have been designed and developed for cancer targeting and imaging *in vivo*.[27] Another emerging modality for molecular imaging is Cerenkov luminescence, which can bridge nuclear imaging with optical imaging via the light emitted during the decay of a radionuclide. Radioactive ^{198}Au-doped Au nanostructures have been designed with different shapes and used for breast cancer imaging *in vivo*.[28,29] These kinds of AuNPs possess great potentials to serve as new platforms for multimodality imaging.

3. Therapy with Gold Nanoparticles

3.1. *AuNPs-based cancer phototherapy*

From a therapeutic perspective, AuNPs have shown promising results in a variety of cancer treatment through photodynamic/photothermal therapy (PDT/PPT) and delivering drugs. PDT is an extraordinary theranostic modality for various malignant tumors. It utilizes reaction between photosensitizer and oxygen presented in tissues to generate reactive oxygen species for effective treatment upon the irradiation of light.[30] However, the PDT efficacy of solid tumors has been largely limited by the depletion and deficiency of tissue oxygen when tumors are large enough to have hypoxic centers or their blood flows are disrupted. PTT can generate heat for therapeutic purposes without requiring the presence of oxygen. High enough concentrations of AuNPs as exogenous photosensitizers are noninvasively delivered to cancer cells and generate localized heat so as to damage tumor regions. The electrons in AuNPs absorb the incoming visible or near infrared reflection (NIR) range of light and achieve a higher energy state then subsequently go back to the stable ground state with heat production. AuNPs mediated PPT has been used widely as a preliminary treatment to

melanoma, epithelial, breast and colon cancer cells both *in vitro* and *in vivo*.[31–33] Controlled surface engineering of AuNPs using ligands of tumor biomarkers and HIV Tat 49–57 which has a membrane translocation domain and a nuclear localization sequence have been successfully used to irreparably damage DNA of cancer cells via directly heating their nucleus through PPT.[34] In addition, noninvasive radiofrequency field (RF, 13.56 MHz) as an energy source can also induce heat of AuNPs within cancer cells for hepatocellular cancer therapy *in vitro*.[35] Systemically administrations of AuNPs have shown their low cytotoxicity and high biocompatibility during tumor treatment. Thus, AuNPs mediated PTT has great potential to become a relatively safe method for future clinical treatment of cancer.

A monolayer of assembled AuNPs loading chlorin e6 (Ce6) are effective multifunctional photosensitizers which can be excited by the illumination of 671 nm laser for simultaneous synergistic PDT/PTT treatment of breast cancer *in vitro* and *in vivo*. The heating effect releases the encapsulated Ce6 molecules from fabricated AuNPs and significantly increases the accumulation of Ce6 and assembled AuNPs in cancer cells for visualizing tumor tissues through thermal, fluorescence and PA signals separately.[36] It is a typical strategy and potential medical model using the optical properties of AuNPs for cancer theranostics.

3.2. *AuNPs-based gene therapy*

Most of the AuNPs used for tumor diagnosis are also suitable for cancer therapy once carrying molecules are able to repress the growth or metastasis of tumor cells. Compare to other NPs, the straightforward synthesis, large surface area, low toxicity and flexible surface chemistry of AuNPs make them suitable for taking more genes to cells than other NPs.[37] Oligonucleotide decorated AuNPs have been used as intracellular gene regulation agents for controlling the protein expression in cells through conjugation or ionic complexation to various nucleic acids, such as DNA, short hairpin RNA (shRNA), small interfering RNA (siRNA) and microRNA (miRNA).[38–41] Figure 1 summarizes two common methods to carry various kinds of genes and several release ways. Negatively charged genes can be attached on positively charged AuNPs and cationic linker, peptide, shell conjugated AuNPs.

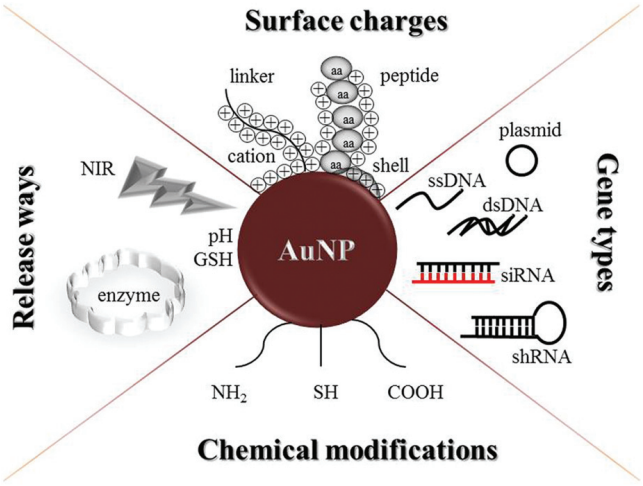

Fig. 1. Cationic surface charges and chemical modifications of AuNPs are adapted to deliver therapeutic genes by NIR light, enzymatic lysis and microenvironment changes of tumor cells for cancer theranostics.

Moreover, the chemical modifications of AuNPs with amino, thiol and carboxyl are able to accept the corresponding decorated genes. With a high affinity for biomolecules, the chemically functionalized AuNPs with alkyl-thiol-terminated oligonucleotides are stable in saline solution and bind their complementary nucleic acids specifically. The mRNA of pro-apoptotic factor BAX hybridized to thiolated αRNA I which chemically attached to the AuNPs for facilitating mRNA transfection into HeLa cells effectively and consequently inhibiting tumor growth *in vitro* and *in vivo*.[42] Ultrasmall AuNPs (2 nm) as carriers for nuclear delivery of triplex-forming oligonucleotides which bind to the promoter of protooncogene c-myc for reducing the generations of c-myc RNA and c-myc protein, subsequently reduce the viability of MCF-7 breast cancer cells.[43] It also demonstrated that NPs smaller than 10 nm could enter the nucleus for intranuclear delivery and therapy, whereas larger ones were found only in the cytoplasm. Although all kinds of polymer-coated AuNPs have been developed to load more nucleotides or to deliver combinations of genetic therapies to cancer cells,[44] there are still rare examples of using AuNPs-oligonucleotides conjugates for gene theranostics *in vivo*. It is partly because of instability of nucleic acid, especially for RNA. At least 9.5 nm of the conjugation distance between siRNAs and AuNPs is required for RNAi-mediated gene silencing. However, it is too hard to keep RNAs extended away from the surface

of AuNPs for a suitable distance during treatment *in vivo*. To overcome these problems, the complicated structures of AuNPs which can completely protect siRNA against enzymatic degradation have been designed.[45] Most of the passive release systems of oligonucleotides and other molecules are based on the cell microenvironments including pH change and different glutathione concentrations.[46] The release of molecules using AuNPs can also be controlled by NIR light illumination and enzymatic hydrolysis, which is an interesting way to achieve local cancer treatment by nontargeting AuNPs.[47]

3.3. *AuNPs-based drug delivery*

Delivery of functional proteins inside malignant cells has been limited by the enzymatic digestion and poor permeability through the cell membrane.[48] The tunability and high surface area of AuNPs provide an excellent platform to attach drugs for controlled and sustained release. AuNPs coated with polyethylene glycol (PEG) have been used to load tumor necrosis factor-alpha to delay tumor growth *in vitro* and *in vivo*.[49] AuNPs functionalized with chemotherapeutic drugs also have been treated as cancer fighters. There are still two kinds of AuNPs-based drug carriers that can be used for tumor therapy, and one of them is surface modified by cationic polymers which load more anionic drugs via electrostatic interactions or chemical conjugations.[50] However, some of chemotherapeutics are too toxic to use as the surface functionalizing molecules which expose to all cells. Therefore, the other remedial delivery way is forming AuNP complexes with encapsulated drugs, which can protect drugs from the enzymatic degradation during intravenous injection as well as effectively prevent the severe damages to normal tissues when chemotherapeutic drugs are fabricated.[51] Surface of AuNPs engineered with cancer targeting molecules may provide specific delivery of drugs to tumor tissues.[52] Selectively targeting cancer cells which with more special receptors is the most predominant factor for precise chemotherapy. Usually, strategy for active targeting of tumors is decorating AuNPs with special ligands which target surface membrane proteins and biomarkers only expressed or overexpressed in cancer cells. The widely used functionalized parts include aptamers, special antibodies and molecules bonding to typical biomarkers of tumors. These molecules are the prerequisites for targeted cancer theranostics by AuNPs taking with chemotherapeutic drugs.

Aptamers are single stranded DNA or RNA molecules with particular secondary or tertiary conformations which facilitate binding suitable targets with high selectivity and affinity. AS1411 as a frequently used G-rich aptamer shows strong affinity to the protein nucleolin which is commonly overexpressed on the surface of malignant cells. AS1411 functionalized AuNPs have been applied to targeted deliver chemotherapeutic doxorubicin in tumor tissues where exist higher concentrations of reductive agents such as glutathione than normal ones for increasing release of drugs, and subsequently reducing cell viability of breast cancer, cervical cancer and uveal melanoma cell lines.[53,54] Anti-His and anti-GST aptamers conjugated to citrate reduced AuNPs are able to selectively deliver various His or GST tagged proteins into varieties of cell types *in vitro* and *in vivo* for protein based tumor treatment.[55] Meanwhile, the aptamer functionalized platform for drug delivery still can combine with other methods to improve the efficacy of chemotherapy.

The expression of some antigens is important in cancers, and then antibodies functionalized AuNPs can be used for targeted cancer theranostics. Recombinant Protein-G was PEGylated to immobilize anti-human EGFR 2 (HER-2) immunoglobulins on AuNPs by the Fc region for efficiently targeting and ablating HER-2 overexpressed breast cancer cells *in vitro*.[56] AuNPs coated with Thomsen Friedenreich antigens which primarily exist on the surface of carcinoma cells has been shown to interact with anti-apoptotic galectin-3 and subsequently inhibit lymphoma cell growth.[57] Quantities of receptors as the biomarkers of tumor tissues specially explored on cell surface can be applied to targeted drug delivery.

Moreover, small molecules such as peptides, growth factors, receptor ligands and so on functionalized AuNPs taking with anti-tumor drugs are also fabricated for local cancer treatment. Glutathione-stabilized AuNPs demonstrate their potential for specifically delivering platinum (IV) drug functionalized with the neuropilin-1 receptor targeting peptide to prostate cancer cells *in vitro*, leading to enhanced cellular uptake level and cell toxicity.[58] Similarly, integrin, HER-2, EGFR and receptors of folate and transferrin are all specially expressed or overexpressed in many tumor types.

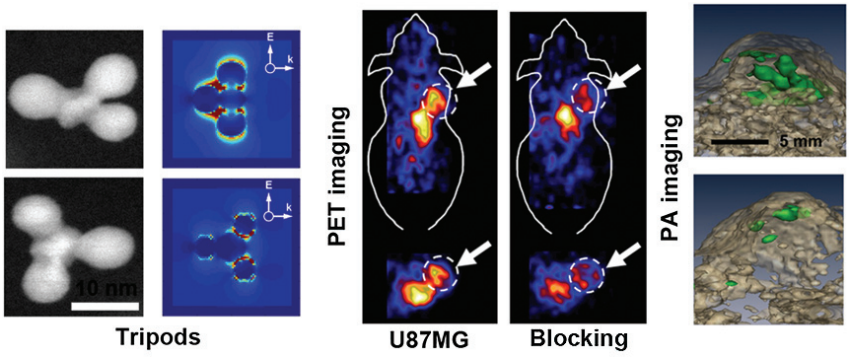

Fig. 2. Structure of gold-based tripod and their PET and PA imaging of xenografted animals. Scanning transmission electron microscope images of tripod-T (A) and tripod-A (B) are definitively identified. Polarization dependence of the average electric field intensity of them localized the edges of tripods and the junctions between two Au−Au NPs as enhanced fields separately in (C) and (D). Small animal PET and PA images of intravenous injected tripods which functionalized ligands of cancer biomarkers (E, G) and blockings (F, H) in mice bearing the U87MG human glioblastoma are shown respectively [Adapted from Cheng, K and reproduced with permission of ACS Publications].[65]

Therefore, RGD (Arg–Gly–Asp) peptide, specific affibodies, folic acid and transferrin can be used as the ligands to functionalize AuNPs separately for targeted drug delivery.[59,60] However, most of these platforms are just widely applied to diagnose cancer through multimodal imaging.[61] Using them as drug carriers is a further way for targeted cancer treatment.

4. Size and Shape of Gold Nanoparticle

For tumor theranostics, the internalized number and time of AuNPs are related to the nanometric size and their shapes. Nanospheres, nanoshells, nanocages and nanorods are commonly used gold nanoshapes in which sizes lie in the ranges of 2–100 nm, 10–200 nm, 10–150 nm and different lengths, respectively. Gold nanospheres are also referred as gold colloids and can be synthesized by controlled reduction of $HAuCl_4$ solution using reducing agent. Gold nanoshell or generally called nanoshell plasmon usually have a dielectric core covered by thin gold shell over it. Gold nanocages are AuNPs which consist of hollow interiors and porous walls. All of them are ideal candidates for cancer theranostic as well as gold nanorods due to their remarkable sets of optical, chemical and physical properties.[62] On the other hand, altering the size and shape of AuNPs to form anisotropic structures can easily tune their localized surface plasmon resonance (LSPR) to the transparent window of soft tissues in the NIR region. Hence, different sizes and shapes of AuNPs provide various photothermal destruction during cancer treatment.[63] Cancer seeking molecules conjugated gold nanorods are potential PA targeting imaging agents for cancer detection *in vivo* because their strong absorption of light and characters of plasmon resonance absorption and scatter in NIR.[64,65] Recently, a novel colloidal hybrid nanostructure with gold-based tripod architecture was designed and prepared for better targeted molecular PA imaging of tumor tissues *in vivo*.[65] Figure 2 showed the structure of gold-based tripod and their uses in PET and PA imaging of xenografted animals. When drug-loaded gold nanostructures are constructed, these systems are extraordinary suitable for cancer theranostics.

5. Conclusion

AuNPs as an ideal platform of molecular nanoprobes and efficient drug carriers for cancer theranostics have been researched and developed for a long time. High surface/volume ratios, low inherent toxicity and facile surface engineering of them present tremendous opportunities in biomedical applications. The electronic and optical properties of AuNPs are extremely powerful tools to detect tumor tissues and practice phototherapy. Targeted ligands functionalized AuNPs efficiently deliver various genes and drugs to cancer cells for precise treatment. Theranostic AuNPs combine diagnosis with therapy of malignant cells in the same platform for convenient application *in vitro* and *in vivo*. However, the long-term cytotoxicity and immune

response existed in tumor treatment using AuNPs are still unsolved problems. Thus, improving functional design and exploiting new structure of AuNPs remains essential for cancer theranostics.

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References

1. Y. Choi, Y. Cho, M. Kim, R. Grailhe, R. Song, "Fluorogenic quantum dot-gold nanoparticle assembly for beta secretase inhibitor screening in live cell," *Anal. Chem.* **84**, 8595–8601 (2012).
2. H. Mollasalehi, R. Yazdanparast, "Non-crosslinking gold nanoprobes for detection of nucleic acid sequence-based amplification products," *Anal. Biochem.* **425**, 91–95 (2012).
3. T. Špringer, J. Homola, "Biofunctionalized gold nanoparticles for spr-biosensor-based detection of cea in blood plasma," *Anal. Bioanal. Chem.* **404**, 2869–2875 (2012).
4. D. T. Nguyen, D.-J. Kim, K.-S. Kim, "Controlled synthesis and biomolecular probe application of gold nanoparticles," *Micron* **42**, 207–227 (2011).
5. S. Keren, C. Zavaleta, Z. Cheng, A. de la Zerda, O. Gheysens, S. S. Gambhir, "Noninvasive molecular imaging of small living subjects using raman spectroscopy," *Proc. Natl. Acad. Sci. USA* **105**, 5844–5849 (2008).
6. A. S. Thakor, R. Luong, R. Paulmurugan, F. I. Lin, P. Kempen, C. Zavaleta, P. Chu, T. F. Massoud, R. Sinclair, S. S. Gambhir, "The fate and toxicity of raman active silica-gold nanoparticles in mice," *Sci. Translat. Med.* **3**, doi:10.1126/scitranslmed.3001963 (2011).
7. J. V. Jokerst, Z. Miao, C. Zavaleta, Z. Cheng, S. S. Gambhir, "Affibody-functionalized gold–silica nanoparticles for raman molecular imaging of the epidermal growth factor receptor," *Small (Weinheim an Der Bergstrasse, Germany)* **7**, 625–633 (2011).
8. S.-P. Lee, "Non-alcoholic fatty liver disease, a marker of subclinical atherosclerosis applicable only to metabolic syndrome?: Time to organize the connection between metabolism and atherosclerosis," *J. Cardiovasc. Ultrasound* **20**, 124–125 (2012).
9. S. Lee, H. Chon, J. Lee, J. Ko, B. H. Chung, D. W. Lim, J. Choo, "Rapid and sensitive phenotypic marker detection on breast cancer cells using surface-enhanced raman scattering (sers) imaging," *Biosens. Bioelectron.* **51**, 238–243 (2014).
10. K. Saha, S. S. Agasti, C. Kim, X. Li, V. M. Rotello, "Gold nanoparticles in chemical and biological sensing," *Chem. Rev.* **112**, 2739–2779 (2012).
11. X. Wang, Y. Xia, Y. Liu, W. Qi, Q. Sun, Q. Zhao, B. Tang, "Dual-luminophore-labeled gold nanoparticles with completely resolved emission for the simultaneous imaging of mmp-2 and mmp-7 in living cells under single wavelength excitation," *Chem. A Eur. J.* **18**, 7189–7195 (2012).
12. R. A. Petros, J. M. DeSimone, "Strategies in the design of nanoparticles for therapeutic applications," *Nat. Rev. Drug Discov.* **9**, 615–627 (2010).
13. H. Lusic, M. W. Grinstaff, "X-ray-computed tomography contrast agents," *Chem. Rev.* **113**, 1641–1666 (2013).
14. K. B. Ghaghada, C. T. Badea, L. Karumbaiah, N. Fettig, R. V. Bellamkonda, G. A. Johnson, A. Annapragada, "Evaluation of tumor microenvironment in an animal model using a nanoparticle contrast agent in computed tomography imaging," *Acad. Radiol.* **18**, 20–30 (2011).
15. J. F. Hainfeld, M. J. O'Connor, F. A. Dilmanian, D. N. Slatkin, D. J. Adams, H. M. Smilowitz, "Micro-ct enables microlocalisation and quantification of her2-targeted gold nanoparticles within tumour regions," *Br. J. Radiol.* **84**, 526–533 (2011).
16. H. Wang, L. Zheng, C. Peng, M. Shen, X. Shi, G. Zhang, "Folic acid-modified dendrimer-entrapped gold nanoparticles as nanoprobes for targeted ct imaging of human lung adencarcinoma," *Biomaterials* **34**, 470–480 (2013).
17. T. Reuveni, M. Motiei, Z. Romman, A. Popovtzer, R. Popovtzer, "Targeted gold nanoparticles enable molecular ct imaging of cancer: An in vivo study," *Int. J. Nanomed.* **6**, 2859–2864 (2011).
18. Q. Chen, H. Wang, H. Liu, S. Wen, C. Peng, M. Shen, G. Zhang, X. Shi, "Multifunctional dendrimer-entrapped gold nanoparticles modified with rgd peptide for targeted computed tomography/magnetic resonance dual-modal imaging of tumors," *Anal. Chem.* **87**, 3949–3956 (2015).
19. T. Curry, R. Kopelman, M. Shilo, R. Popovtzer, "Multifunctional theranostic gold nanoparticles for targeted ct imaging and photothermal therapy," *Contrast Media Mol. Imag.* **9**, 53–61 (2014).
20. J. Li, Y. Hu, J. Yang, P. Wei, W. Sun, M. Shen, G. Zhang, X. Shi, "Hyaluronic acid-modified fe3o4@au core/shell nanostars for multimodal imaging and photothermal therapy of tumors," *Biomaterials* **38**, 10–21 (2015).
21. Q. Chen, K. Li, S. Wen, H. Liu, C. Peng, H. Cai, M. Shen, G. Zhang, X. Shi, "Targeted ct/mr dual mode

imaging of tumors using multifunctional dendrimer-entrapped gold nanoparticles," *Biomaterials* **34**, 5200–5209 (2013).

22. K. Li, S. Wen, A. C. Larson, M. Shen, Z. Zhang, Q. Chen, X. Shi, G. Zhang, "Multifunctional dendrimer-based nanoparticles for in vivo mr/ct dual-modal molecular imaging of breast cancer," *Int. J. Nanomed.* **8**, 2589–2600 (2013).
23. L. Karmani, D. Labar, V. Valembois, V. Bouchat, P. G. Nagaswaran, A. Bol, J. Gillart, P. Levêque, C. Bouzin, D. Bonifazi, C. Michiels, O. Feron, V. Grégoire, S. Lucas, T. V. Borght, B. Gallez, "Antibody-functionalized nanoparticles for imaging cancer: Influence of conjugation to gold nanoparticles on the biodistribution of 89zr-labeled cetuximab in mice," *Contrast Media Mol. Imag.* **8**, 402–408 (2013).
24. L. Karmani, V. Bouchat, C. Bouzin, P. Levêque, D. Labar, A. Bol, G. Deumer, R. Marega, D. Bonifazi, V. Haufroid, C. Michiels, V. Grégoire, O. Feron, S. Lucas, T. Vander Borght, B. Gallez, "89zr-labeled anti-endoglin antibody-targeted gold nanoparticles for imaging cancer: Implications for future cancer therapy," *Nanomedicine* **9**, 1923–1937 (2014).
25. Y. Zhao, D. Sultan, L. Detering, H. Luehmann, Y. Liu, "Facile synthesis, pharmacokinetic and systemic clearance evaluation, and positron emission tomography cancer imaging of 64cu-au alloy nanoclusters," *Nanoscale* **6**, 13501–13509 (2014).
26. Y. Zhao, D. Sultan, L. Detering, S. Cho, G. Sun, R. Pierce, K. L. Wooley, Y. Liu, "Copper-64-alloyed gold nanoparticles for cancer imaging: Improved radiolabel stability and diagnostic accuracy," *Angew. Chem. Int. Ed.* **53**, 156–159 (2014).
27. H. Groult, J. Ruiz-Cabello, J. Pellico, A. V. Lechuga-Vieco, R. Bhavesh, M. Zamai, E. Almarza, I. Martín-Padura, E. Cantelar, M. P. Martínez-Alcázar, F. Herranz, "Parallel multifunctionalization of nanoparticles: A one-step modular approach for in vivo imaging," *Bioconjugate Chem.* **26**, 153–160 (2015).
28. K. C. L. Black, Y. Wang, H. P. Luehmann, X. Cai, W. Xing, B. Pang, Y. Zhao, C. S. Cutler, L. V. Wang, Y. Liu, Y. Xia, "Radioactive 198au-doped nanostructures with different shapes for in vivo analyses of their biodistribution, tumor uptake, and intratumoral distribution," *ACS Nano* **8**, 4385–4394 (2014).
29. Y. Wang, Y. Liu, H. Luehmann, X. Xia, D. Wan, C. Cutler, Y. Xia, "Radioluminescent gold nanocages with controlled radioactivity for real-time in vivo imaging," *Nano Lett.* **13**, 581–585 (2013).
30. K. Y. Choi, G. Liu, S. Lee, X. Chen, "Theranostic nanoplatforms for simultaneous cancer imaging and therapy: Current approaches and future perspectives," *Nanoscale* **4**, 330–342 (2012).
31. H.-C. Huang, S. Barua, G. Sharma, S. K. Dey, K. Rege, "Inorganic nanoparticles for cancer imaging and therapy," *J. Control. Release* **155**, 344–357 (2011).
32. J. Choi, J. Yang, D. Bang, J. Park, J.-S. Suh, Y.-M. Huh, S. Haam, "Targetable gold nanorods for epithelial cancer therapy guided by near-ir absorption imaging," *Small* **8**, 746–753 (2012).
33. S.-H. Seo, B.-M. Kim, A. Joe, H.-W. Han, X. Chen, Z. Cheng, E.-S. Jang, "Nir-light-induced surface-enhanced raman scattering for detection and photothermal/photodynamic therapy of cancer cells using methylene blue-embedded gold nanorod@sio2 nanocomposites," *Biomaterials* **35**, 3309–3318 (2014).
34. N. Jiménez-Mancilla, G. Ferro-Flores, C. Santos-Cuevas, B. Ocampo-García, M. Luna-Gutiérrez, E. Azorín-Vega, K. Isaac-Olivé, M. Camacho-López, E. Torres-García, "Multifunctional targeted therapy system based on 99mtc/177lu-labeled gold nanoparticles-tat(49–57)-lys3-bombesin internalized in nuclei of prostate cancer cells," *J. Label. Comp. Radiopharm.* **56**, 663–671 (2013).
35. M. Raoof, S. J. Corr, W. D. Kaluarachchi, K. L. Massey, K. Briggs, C. Zhu, M. A. Cheney, L. J. Wilson, S. A. Curley, "Stability of antibody-conjugated gold nanoparticles in the endolysosomal nanoenvironment: Implications for noninvasive radiofrequency-based cancer therapy," *Nanomed. Nanotechnol. Biol. Med.* **8**, 1096–1105 (2012).
36. J. Lin, S. Wang, P. Huang, Z. Wang, S. Chen, G. Niu, W. Li, J. He, D. Cui, G. Lu, X. Chen, Z. Nie, "Photosensitizer-loaded gold vesicles with strong plasmonic coupling effect for imaging-guided photothermal/photodynamic therapy," *ACS Nano* **7**, 5320–5329 (2013).
37. V. Biju, "Chemical modifications and bioconjugate reactions of nanomaterials for sensing, imaging, drug delivery and therapy," *Chem. Soc. Rev.* **43**, 744–764 (2014).
38. E. Jeong, G. Jung, C. Hong, H. Lee, "Gold nanoparticle (aunp)-based drug delivery and molecular imaging for biomedical applications," *Arch. Pharm. Res.* **37**, 53–59 (2014).
39. H. Jaganathan, S. Mitra, S. Srinivasan, B. Dave, B. Godin, "Design and in vitro evaluation of layer by layer sirna nanovectors targeting breast tumor initiating cells," *PLoS ONE* **9**, e91986 (2014).
40. J. Conde, A. Ambrosone, V. Sanz, Y. Hernandez, V. Marchesano, F. Tian, H. Child, C. C. Berry, M. R. Ibarra, P. V. Baptista, C. Tortiglione, J. M. de la Fuente, "Design of multifunctional gold

nanoparticles for in vitro and in vivo gene silencing," *ACS Nano* **6**, 8316–8324 (2012).
41. A. Ekin, O. F. Karatas, M. Culha, M. Ozen, "Designing a gold nanoparticle-based nanocarrier for microrna transfection into the prostate and breast cancer cells," *J. Gene Med.* **16**, 331–335 (2014).
42. J.-H. Yeom, S.-M. Ryou, M. Won, M. Park, J. Bae, K. Lee, "Inhibition of xenograft tumor growth by gold nanoparticle-DNA oligonucleotide conjugates-assisted delivery of bax mrna," *PLoS ONE* **8**, e75369 (2013).
43. S. Huo, S. Jin, X. Ma, X. Xue, K. Yang, A. Kumar, P. C. Wang, J. Zhang, Z. Hu, X.-J. Liang, "Ultrasmall gold nanoparticles as carriers for nucleus-based gene therapy due to size-dependent nuclear entry," *ACS Nano* **8**, 5852–5862 (2014).
44. C. J. Bishop, S. Y. Tzeng, J. J. Green, "Degradable polymer-coated gold nanoparticles for co-delivery of DNA and sirna," *Acta Biomater.* **11**, 393–403 (2015).
45. L. Han, J. Zhao, X. Zhang, W. Cao, X. Hu, G. Zou, X. Duan, X.-J. Liang, "Enhanced sirna delivery and silencing gold–chitosan nanosystem with surface charge-reversal polymer assembly and good biocompatibility," *ACS Nano* **6**, 7340–7351 (2012).
46. A. Llevot, D. Astruc, "Applications of vectorized gold nanoparticles to the diagnosis and therapy of cancer," *Chem. Soc. Rev.* **41**, 242–257 (2012).
47. Y. Cheng, T. L. Doane, C.-H. Chuang, A. Ziady, C. Burda, "Near infrared light-triggered drug generation and release from gold nanoparticle carriers for photodynamic therapy," *Small* **10**, 1799–1804 (2014).
48. S. Rana, A. Bajaj, R. Mout, V. M. Rotello, "Monolayer coated gold nanoparticles for delivery applications," *Adv. Drug Deliv. Rev.* **64**, 200–216 (2012).
49. A. S. Thakor, J. Jokerst, C. Zavaleta, T. F. Massoud, S. S. Gambhir, Gold nanoparticles: A revival in precious metal administration to patients," *Nano Lett.* **11**, 4029–4036 (2011).
50. N. C. Bigall, A. Curcio, M. P. Leal, A. Falqui, D. Palumberi, R. Di Corato, E. Albanesi, R. Cingolani, T. Pellegrino, "Magnetic nanocarriers with tunable ph dependence for controlled loading and release of cationic and anionic payloads," *Adv. Mat.* **23**, 5645–5650 (2011).
51. C. M. Dawidczyk, C. Kim, J. H. Park, L. M. Russell, K. H. Lee, M. G. Pomper, P. C. Searson, "State-of-the-art in design rules for drug delivery platforms: Lessons learned from fda-approved nanomedicines," *J. Control. Release* **187**, 133–144 (2014).
52. S. C. Coelho, S. Rocha, P. Juzenas, P. Sampaio, G. M. Almeida, F. S. Silva, M. C. Pereira, M. A. N. Coelho, "Gold nanoparticle delivery-enhanced proteasome inhibitor effect in adenocarcinoma cells," *Expert Opin. Drug Deliv.* **10**, 1345–1352 (2013).
53. A. Latorre, C. Posch, Y. Garcimartin, A. Celli, M. Sanlorenzo, I. Vujic, J. Ma, M. Zekhtser, K. Rappersberger, S. Ortiz-Urda, A. Somoza, "DNA and aptamer stabilized gold nanoparticles for targeted delivery of anticancer therapeutics," *Nanoscale* **6**, 7436–7442 (2014).
54. Y.-S. Shiao, H.-H. Chiu, P.-H. Wu, Y.-F. Huang, "Aptamer-functionalized gold nanoparticles as photoresponsive nanoplatform for co-drug delivery," *ACS Appl. Mat. Interfaces* **6**, 21832–21841 (2014).
55. S.-M. Ryou, J.-H. Yeom, H. J. Kang, M. Won, J.-S. Kim, B. Lee, M.-J. Seong, N.-C. Ha, J. Bae, K. Lee, "Gold nanoparticle–DNA aptamer composites as a universal carrier for in vivo delivery of biologically functional proteins," *J. Control. Release* **196**, 287–294 (2014).
56. X. Sun, G. Zhang, R. S. Keynton, M. G. O'Toole, D. Patel, A. M. Gobin, "Enhanced drug delivery via hyperthermal membrane disruption using targeted gold nanoparticles with pegylated protein-g as a cofactor," *Nanomed. Nanotechnol. Biol. Med.* **9**, 1214–1222 (2013).
57. S. Biswas, S. H. Medina, J. J. Barchi Jr., "Synthesis and cell-selective antitumor properties of amino acid conjugated tumor-associated carbohydrate antigen-coated gold nanoparticles," *Carbohydrate Res.* **405**, 93–101 (2015).
58. A. Kumar, S. Huo, X. Zhang, J. Liu, A. Tan, S. Li, S. Jin, X. Xue, Y. Zhao, T. Ji, L. Han, H. Liu, X. Zhang, J. Zhang, G. Zou, T. Wang, S. Tang, X.-J. Liang, "Neuropilin-1-targeted gold nanoparticles enhance therapeutic efficacy of platinum(iv) drug for prostate cancer treatment," *ACS Nano* **8**, 4205–4220 (2014).
59. M. S. Mohamed, S. Veeranarayanan, A. C. Poulose, Y. Nagaoka, H. Minegishi, Y. Yoshida, T. Maekawa, D. S. Kumar, "Type 1 ribotoxin-curcin conjugated biogenic gold nanoparticles for a multimodal therapeutic approach towards brain cancer," *Biochim. Biophys. Acta Gen. Subj.* **1840**, 1657–1669 (2014).
60. W. Pan, H. Yang, T. Zhang, Y. Li, N. Li, B. Tang, "Dual-targeted nanocarrier based on cell surface receptor and intracellular mrna: An effective strategy for cancer cell imaging and therapy," *Anal. Chem.* **85**, 6930–6935 (2013).
61. Y. Wang, Z. Miao, G. Ren, Y. Xu, Z. Cheng, "A novel affibody bioconjugate for dual-modality imaging of ovarian cancer," *Chem. Commun.* **50**, 12832–12835 (2014).
62. M. S. Khan, G. D. Vishakante, H. Siddaramaiah, "Gold nanoparticles: A paradigm shift in biomedical applications," *Adv. Colloid Interface Sci.* **199–200**, 44–58 (2013).

63. Y. Wang, K. C. L. Black, H. Luehmann, W. Li, Y. Zhang, X. Cai, D. Wan, S.-Y. Liu, M. Li, P. Kim, Z.-Y. Li, L. V. Wang, Y. Liu, Y. Xia, "Comparison study of gold nanohexapods, nanorods, and nanocages for photothermal cancer treatment," *ACS Nano* **7**, 2068–2077 (2013).
64. Z. Heidari, R. Sariri, M. Salouti, "Gold nanorods-bombesin conjugate as a potential targeted imaging agent for detection of breast cancer," *J. Photochem. Photobiol. B: Biol.* **130**, 40–46 (2014).
65. K. Cheng, S.-R. Kothapalli, H. Liu, A. L. Koh, J. V. Jokerst, H. Jiang, M. Yang, J. Li, J. Levi, J. C. Wu, S. S. Gambhir, Z. Cheng, "Construction and validation of nano gold tripods for molecular imaging of living subjects," *J. Am. Chem. Soc.* **136**, 3560–3571 (2014).