Gold nanostar-based complexes applied for cancer theranostics

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Abstract
Cancer remains a major health problem that plagues human beings, calling widespread attention to develop novel theranostics to achieve sensitive diagnosis and efficient therapy. Multifunctional nanomedicine that can integrate diagnosis with treatment formulations has been emerging as a powerful strategy to overcome the current drawbacks in conventional clinical cancer treatments. Due to the good biocompatibility, easy surface modification, surface-enhanced Raman spectroscopy (SERS)/computed tomography (CT)/photoacoustic (PA) imaging properties, and exceptional photothermal performance of gold nanostars (AuNSs), various AuNS-based complexes or nanohybrids including metal compound/AuNSs, SiO2/AuNSs, polymer/AuNSs, and dendrimer/AuNSs, and so forth have been developed, holding great blueprint in cancer theranostics. Herein, we concisely review the recent progresses in the intriguing design of AuNS-based nanoplatforms, and their applications in bioimaging, therapy and imaging-guided cancer treatment, and clarify the possible future perspectives for the design of AuNS-facilitated cancer theranostics.

KEYWORDS
cancer theranostics, gold nanostars, imaging, nanohybrids, therapy

1 | INTRODUCTION

According to the latest statistics released by the International Agency for Research on Cancer, there were more than 19.0 million incident cases and more than 9.9 million deaths were caused by cancer worldwide in 2020. With emphatical increase year by year, the number of new cancer cases and deaths worldwide by 2040 may predictably increase to 30.2 and 16.3 million, respectively, which renders cancer a long-lasting health burden for the human beings to overcome. Despite the progresses in medical care and equipment, current clinical trials are still facing problems due to the heterogeneity, metastasis, drug resistance, and complexity of cancer. In recent decades, with
the development of nanotechnology, imaging technology, and novel therapeutics, multimodal imaging-guided combination therapy has been extensively explored and will be expected to promote the innovation in cancer theranostics.1-4 Up to date, a variety of nanomaterials and nanomaterial-based platforms have been researched and applied in cancer theranostics, including metal nanoparticles (NPs),9 liposomes,10 polymeric micelles,11 nanofibers,12 and dendrimers.13

Among these potential candidates, gold nanostars (AuNSs) with unique multiple sharp branches and a high atomic number have been considered as a promising “one-for-all” design for cancer theranostics due to their sensitive surface-enhanced Raman spectroscopy (SERS)/computed tomography (CT)/photoacoustic (PA) imaging, and exceptional photothermal conversion effect.14 The localized surface plasmon resonance (LSPR) property of AuNSs can induce the significantly enhanced absorption and scattering of incident light. On one hand, the Mie scattering of excitation light can be developed for SERS imaging; on the other hand, the energy of the absorbed light can be converted into heat due to the excellent photothermal conversion efficiency of AuNSs, which is the premise for photothermal therapy (PTT) and PA imaging applications.15,16

Through further assembly with other nanomaterials with special functions, multifunctional AuNS-based nanohybrids have been developed as a category of “all-in-one” theranostic design, such as metal compound/AuNSs,17 SiO2/AuNSs,18 polymer/AuNSs,19 dendrimer/AuNSs,20 and others.21 To overcome the resistance encountered in single-mode imaging diagnosis and therapy of cancer, the potential of AuNS-based nanohybrids has been explored to integrate SERS, PA, CT, or magnetic resonance (MR) imaging mode to achieve precision imaging with high sensitivity, outstanding spatial resolution, and high contrast ability, and to realize multimodal imaging-guided combination tumor therapy.22 In addition to the direct thermal ablation of tumors caused by PTT, AuNS-based nanohybrids make it feasible for the expanding vessels and tissues to increase the oxygen content and enhance the accumulation of drugs in solid tumors, thus achieving PTT-enhanced photodynamic therapy (PDT), radiation therapy (RT), and chemotherapy to facilitate the clinical transformation of nanomedicine. Although recent reviews have discussed the synthesis methods of single AuNSs and their biomedical applications, there is still no state-of-art review systematically summarizing the design of AuNS-based nanohybrids for cancer management. Herein, we review the recent key developments of AuNS-based nanohybrids for cancer theranostics (Figure 1), including the synthesis strategies of different kinds of AuNS-based nanohybrids, and their applications in bioimaging, therapy, and imaging-guided treatment of cancer, as well as the possible perspectives for the design of AuNS-based nanohybrids toward cancer theranostics.

2 | SYNTHESIS OF AUNS-BASED NANOHYBRIDS

As the synthesis methods of AuNSs have been reported previously, this review mainly covers the preparation strategies of AuNS-based hybrid nanomaterials. In short, there are currently two main strategies employed to create AuNSs: seed-mediated growth and one-pot method.44 At present, the localized surface plasmon resonance (LSPR) absorption of AuNSs can be tuned by controlling the type and concentration of reactants (seeds or ligands), reaction time, and temperature.44-47 What is worthy to be mentioned here is that the surfactants themselves are generally toxic, largely limiting the biomedical applications of AuNSs.48-50 In 2012, Yuan et al. prepared AuNSs through an improved surfactant-free method using Au seeds.47 Recently, more flexible strategies and various kinds of natural compounds have been integrated to form novel bio-compatible AuNSs.51-56

Hybrid nanomaterials with remarkable and multifunctional properties are able to overcome the drawbacks of single-component materials.57-59 Thereafter, AuNS-based nanohybrids, as a category of “all-in-one” design, not only possess SERS/CT/PA imaging capability and outstanding photothermal conversion efficiency, but also cover the unique physical, chemical, and optical properties of other materials.60,61 By far, the synthesis strategies of AuNS-based nanohybrids are mainly based on the evolution of current preparation method of AuNSs mentioned in Table 1.
### TABLE 1 Summary of recent designs and applications of AuNS-based nanohybrids in cancer theranostics

| Nanohybrids       | Functional compound(s) | Applications                                      | Method            | Cancer                          | Ref.  |
|-------------------|-------------------------|---------------------------------------------------|-------------------|---------------------------------|-------|
| Metal compound/ AuNSs | Fe$_3$O$_4$, FA,        | PA/CT/MR imaging, PTT                             | Hybrid seed       | Xenografted HeLa tumor          | [28]  |
|                   | Fe$_3$O$_4$, HA         | MR/CT imaging, PTT                                | Hybrid seed       | Xenografted HeLa tumor          | [17]  |
| SiO$_2$/AuNSs     | HMS, PFH, PEG           | US/CT/PA imaging, PTT                             | Hybrid seed       | Xenografted C6 tumor            | [29]  |
|                   | mSiO$_2$, DOX, paraffin heneicosane | PTT/chemotherapy                                 | Single seed       | HeLa cells                      | [30]  |
|                   | HMS, Gem, PFH, IGF1     | US/CT/PA imaging, PTT                             | Hybrid seed       | Xenografted AsPC-1 tumor        | [31]  |
|                   | HMS, PEG, RGD           | PTT/chemotherapy                                  | Hybrid seed       | Xenografted U87MG tumor         | [32]  |
| Polymer/AuNSs     | PEG, MTX                | SERS/FTIR imaging, chemotherapy                   | Single seed       | Xenografted Calu-1 tumors       | [33]  |
|                   | Chitosan                | PA imaging, PTT                                   | Template method   | MDA-MB-231 cells                | [34]  |
|                   | HA, DOX, cationic peptide R8, TPP-KLA | PTT/chemotherapy                                 | Seedless synthesis| Orthotopic SCC-7 tumors         | [35]  |
| Liposomes         | paclitaxel, siRNA, COX-2, DG, 9R | PTT/gene/chemotherapy                        | Single seed       | Xenografted HeLa tumors         | [36]  |
|                   |                         |                                                   |                   | PTX-resistant HepG2/PTX cells   | [37]  |
| Dendrimer/AuNSs   | Dendrimer (G3), RGD, siRNA | CT imaging, PTT/gene therapy                   | Single seed       | Xenografted U87MG tumor         | [20]  |
|                   | Dendrimer (G5), Fe$_3$O$_4$ | T$_2$ -MR/PA/CT imaging, PTT/RT                  | Hybrid seed       | Xenografted 4T1 tumors          | [38]  |
| Other AuNSs       | GO, DODAB, DOPE, FA, Krasl | PA imaging, PTT/gene therapy                    | Seedless synthesis| Xenografted Cupan-1 tumors      | [39]  |
|                   | ZIF-8, DOX              | PA imaging, PTT/chemotherapy                      | Template method   | Xenografted H22 tumors          | [22]  |
|                   | ASI411, DOX             | PTT/chemotherapy                                  | Single seed       | Xenografted adriamycin-resistant MCF-7/ADR tumors | [40]  |

Abbreviations: 9R, 9-poly-D-arginine; AuNSs, gold nanostars; BSA, bovine serum albumin; Ce6, chlorin e6; ConA, Concanavalin A; CT, computed tomography; DG, 2-deoxyglucose; DODAB, dimethyldioctadecylammonium bromide; DOPE, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine; DOX, doxorubicin; DTTC, 3,3-diethylthiatricarbocyanine iodide; EDC, 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride; EM, electromagnetic; EPR, enhanced permeability and retention; FA, folic acid; FTIR, Fourier transform infrared; G3, generation 3; G5, generation 5; Gem, gemcitabine; GO, graphene; HA, hyaluronic acid; HEPES, [4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid; HMSs, hollow mesoporous silica nanocapsules; IGF1, insulin-like growth factor-1; Ir, iridium; KrasI, K-Ras gene plasmid; LSPR, localized surface plasmon resonance; MR, magnetic resonance; MSC, mesenchymal stem cells; MSOT, multispectral optoacoustic tomography; MTX, mitoxantrone; NHS, N-hydroxysuccinimide; NK, natural killer; NPs, nanoparticles; PA, photoacoustic; P-AE105, polyetherimide-AE105 peptide; PAMAM, poly(amidoamine); PDA, polydopamine; PDT, photodynamic therapy; PEG, polyethylene glycol; PEI, poly(ethylene imine); PFH, perfluorohexane; PTT, photothermal therapy; PTX-TSL, paclitaxel loaded temperature-sensitive liposomes; RT, radiation therapy; SERRS, surface-enhanced resonance Raman scattering; SERS, surface-enhanced Raman spectroscopy; siCOX-2, siRNA of cyclooxygenase-2; TAT, trans-activating transcriptional activator peptide; TNBC, triple-negative breast cancer; TPP, triphenylphosphine; US, ultrasonic; USIO, ultrasmall iron oxide; ZIF-8, zeolitic imidazolate framework-8.

### 2.1 Seed-mediated growth method

For the fabrication of AuNS-based hybrid materials via the seed method, there are two kinds of seeds that have been generally adopted. One is to obtain the AuNSs through the growth of the Au seeds first, followed by complexation or hybridization with other components. The other way is to first obtain the composite NPs based on Ag or Au as a hybrid seed for subsequent growth of AuNSs. In both cases, the AuNSs or AuNS-based organic/inorganic
or inorganic/inorganic hybrids can be further decorated with polymers or bioactive ingredients to create varying functional nanohybrids for subsequent biomedical applications.

2.1.1 Single seed-mediated growth method

Based on the traditional seed method, further integration and chemical modification of AuNSs with other components leads to the creation of various kinds of AuNS-based nanohybrids, such as metal compound/AuNSs, SiO$_2$/AuNSs, dendrimer/AuNSs, and MOF/AuNSs nanohybrids. For instance, Montoto et al. reported the preparation of mesoporous silica-coated AuNSs. In their work, AuNSs synthesized by a seed growth method were used as cores to synthesize mesoporous silica shell (mSiO$_2$) based on a surfactant-templated synthesis method. The method involved the following steps: adsorption of cationic surfactant micelles onto the surface of AuNSs, hydrolysis of a silica precursor of tetraethoxysilane, condensation of silica oligomers within the surfactant micelles, and deposition of silica shells onto the AuNS surface. Through further loading of doxorubicin (DOX), an anticancer drug, surface functionalization with a monolayer of octadecyltrimethoxysilane and coating with a paraffin shell, the so-called near-infrared (NIR)-stimulated AuNS@mSiO$_2$@DOX@paraffin nanohybrids were formed.

Poly(amidoamine) (PAMAM) dendrimers with the structural similarity to proteins have been paid increasing attention in biomedical applications due to their unique superiorities, such as excellent water solubility, highly branched inner cavities, nonimmunogenicity, and ease of functionalization. To create dendrimer-stabilized AuNSs, AuNSs synthesized by a seed-mediated method were modified with partially thiolated generation 3 (G3) PAMAM dendrimers via Au–S bonding. Interestingly, the G3 PAMAM dendrimers could be used as a linker to modify the AuNSs with cyclic arginine-glycine-aspartic (Arg-Gly-Asp, RGD) through a polyethylene glycol (PEG) spacer via 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC)/N-hydroxysuccinimide (NHS) coupling chemistry. Through an electrostatic interaction at the optimized N/P ratio, siRNA could be compacted with the AuNS/dendrimer nanohybrids.

2.1.2 Hybrid seed-mediated growth method

Instead of using single Au or Ag NPs as seeds to create AuNS nanohybrids, AuNS-based nanohybrids can also be formed using composite NPs as seeds. For instance, our group reported the synthesis of hyaluronic acid (HA)-modified Fe$_3$O$_4$@Au core/shell nanostars (Fe$_3$O$_4$@Au-HA NSs) by the hybrid seed-mediated growth method (Figure 2). Here, the Fe$_3$O$_4$@Ag composite seeds were first obtained by hydrothermal treatment of Fe(II) ions in the presence of preformed polyethyleneimine (PEI)-stabilized Ag NPs, then exposed to an Au growth solution containing HAuCl$_4$, hexadecyl trimethyl ammonium bromide, AgNO$_3$, and ascorbic acid to generate the hybrid Fe$_3$O$_4$@AuNSs. The Fe$_3$O$_4$@AuNSs could be further functionalized with PEI-SH through Au–S bonding and HA through EDC/NHS coupling to form multifunctional Fe$_3$O$_4$@Au-HA NSs with improved stability and targeting specificity for subsequent biomedical applications.

In another work, our group also prepared a hybrid seed of Gd(OH)$_3$@Au NPs through a hydrothermal treatment of Gd(III) in the presence of preformed PEI-entrapped AuNPs (PEI-AuNPs). The formed Gd(OH)$_3$@Au hybrid NPs with a rod shape were used as seeds for further growth of AuNSs in the Au growth solution to lead to the formation of NSs-on-nanorods hybrids. The prepared Gd(OH)$_3$@AuNSs can be controlled in size, shape, composition, and optical properties by adjusting the stabilizer type, reaction time, solvent and molar feeding ratio of Gd/Au.

2.2 One-pot method

As a rapid, simple, and controllable synthesis method, the one-pot method has been widely applied to prepare various AuNS-based nanohybrids. In particular, the one-pot method includes seedless and template synthesis. To be the same to the seed-mediated growth methods, the thus created AuNSs can always be further modified with polymers or be biofunctionalized before biomedical applications.

2.2.1 Seedless synthesis

For seedless synthesis of AuNS-based hybrids with tailored dimensions, shape, and optical properties, a variety of materials including PEG, PEI, lipid, chitosan, polydopamine (PDA), and HA have been selected for the preparation and functionalization of AuNSs to improve their stability, biocompatibility, and targeting specificity. For instance, Chen and coworkers prepared HA- and peptide-modified AuNS nanohybrids (AuNS-pep/DOX@HA) via a seedless synthesis method. In their work, AuNSs created through one-pot reduction of HAuCl$_4$ in 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer at room temperature were linked with both a cysteine-modified peptide (with both units of cationic peptide [R8] and
triphenylphosphine modified α-helical pro-apoptotic peptide [TPP-KLA]) and PEG-SH through Au–S bonding. Through further electrostatic interaction and physical encapsulation, HA and DOX could be coated onto the surface of AuNS nanohybrids and loaded within the particles, respectively, to create multifunctional AuNS-pep/DOX@HA hybrids. Here, HEPES is regarded as both a reducing and shape-directing agent during the synthesis of AuNSs. It should be emphasized that the nitrogen-centered free radicals generated by the piperazine ring in HEPES can reduce Au salt.67

Besides, inorganic components can also be integrated with the AuNSs formed through the same seedless method using HEPES buffer. In a recent work by Jia et al.,39 AuNSs were first formed through seedless synthesis in the HEPES buffer in the presence of graphene (GO), which can serve as a supporting material through physical interactions between Au(III) salt and the oxygenated GO surface groups. The used HEPES was able to control the anisotropic growth of GO@AuNSs that were surface coated with mixed cationic lipids of dimethyl dioctadecylammonium bromide (DODAB), 1,2-dioleoyl-sn-glycerol-3-phosphoethanolamine (DOPE), and folic acid (FA)-modified DOPE. After that, ascorbic acid was used to reduce the component of GO to enhance its photothermal properties for further electrostatic interaction with a gene (targeting G12V mutant K-Ras gene). The functional nanohybrids of GO@AuNS/lipid (rGADA-KrasI) were finally formed for subsequent biomedical applications (Figure 3).

2.2.2 Template method

The template method enables the synthesis of AuNS-based nanohybrids through a green chemistry route, and
is feasible to involve different kinds of naturally occurring polymers with desired biocompatibility. For instance, chitosan, a biopolymer, has been chosen as a template to mediate the synthesis of AuNSs and plays the roles of surface passivation, size control, and shape directing. Recently, Phan et al. studied the influence of pH and chitosan concentration on the one-pot synthesis of AuNSs. The growing process of AuNSs can be divided into two stages: (a) Au(III) ions are quickly reduced to Au seeds by vitamin C, and under an acidic pH condition (pH = 1.0), the protonated polycationic chitosan can bind to the Au seeds and Au salt via a strong electrostatic interaction; (b) under an optimized chitosan concentration, the sharp spikes of Au seeds start to grow at some of the points with loose chitosan contact, whereas the close contact site does not allow the Au spikes to grow, thus facilitating the anisotropic growth of AuNSs.

In addition to chitosan, bovine serum albumin (BSA), with the nontoxic nature and ligand-binding properties has also been used as a template to create AuNSs with improved colloidal stability, biocompatibility, and functionality. For instance, Sasidharan et al. reported a rapid one-pot synthesis method of AuNSs using BSA as a template. The authors found that the net positive charge of BSA at a pH below its isoelectric point (pH = 4.7) facilitated the attachment of Au salt, which was subsequently reduced to Au(0) using ascorbic acid. In addition, the growth of multiple sharp branches on the surface of AuNSs using BSA was determined to be a pH-dependent process, where the stretching of the α-helices of BSA at a low pH and their transformation to the β-sheet conformer were regarded as the favorable condition for the growth of the multiple sharp branches of AuNSs.

### 3 AUNS-BASED NANOHYBRIDS FOR CANCER NANOMEDICINE APPLICATIONS

With the mutual interaction between AuNSs and neighboring materials at the nanoscale, AuNS-based nanohybrids possess complex interfacial behaviors such as electron transfer and near-field enhancement, leading to potential synergistic performance enhancement compared to the simple sum of the isolated single components. In addition, functionalized AuNS-based nanohybrids formed via ligand exchange based on the Au–S bonding (PEG, PEL, deoxyribose nucleic acid, peptide, or dendrimer) or physical interaction (drugs, photosensitizer, or lipids) provide many possibilities for imaging, therapy, and theranostics of cancer.

#### 3.1 Multimodal imaging

Multimodal imaging endows complementary and precision cancer diagnosis, and has become a research hotspot for guiding tumor therapy. Due to the unique composition and structure, AuNSs have been regarded as a great multimodal imaging agent for tumor diagnosis. As early as 2016, Tian et al. synthesized the mitoxantrone (MTX)-loaded AuNSs (MTX-PEG-AuNSs) for multimodal SERS/Fourier transform infrared (FTIR) spectroscopy imaging of xenograft Calu-1 tumors. The MTX-PEG-AuNSs with desirable biocompatibility could be applied to monitor the real-time MTX release throughout the cell cycle and in vivo metabolism.

In another work, Neuschmelting et al. reported a core-shell AuNS-based nanohybrid composed of an AuNS core and a resonant Raman reporter IR780-embedded silica shell that was functionalized with methoxy-terminated PEG. The created nanohybrids were used for dual-modality surface-enhanced resonance Raman scattering (SERRS) and multispectral optoacoustic tomography (MSOT) imaging of glioblastoma (GBM) (Figure 4). The MSOT with a high depth penetration enabled a real-time stereo-deep GBM imaging, but exhibited a low sensitivity to the GBM marginal sites. However, the SERRS enabled extremely sensitive high-resolution surface detection to differentiate the true microscopic diffuse infiltrations of GBM. Therefore, the combined SERRS and MSOT dual-mode imaging can significantly improve the imaging precision of GBM.

#### 3.2 Combination therapy

PTT can generate topical hyperthermia (above 42°C) under laser irradiation to kill cancer cells via photothermal ablation. Due to the superiority of hyperthermia, high spatial-temporal precision, deep tissue penetration, little side effects, and minor invasiveness, PTT has been selected as a potential clinical treatment method under laser light, especially in the NIR-II window (1000–1500 nm). AuNSs with a strong NIR-II absorption and high photothermal conversion efficiency are excellent candidates for efficient PTT of tumors.

In general, single-mode therapy cannot achieve the expected therapeutic outcome. Hence, it is important to develop nanohybrids for combination tumor therapy, which integrates complementary advantages of different therapy modes. Various AuNS-based nanohybrids have been developed for combination tumor therapy with formidable antitumor capabilities. For instance, Montoto et al. developed an NIR-stimulated
AuNSs@mSiO$_2$@DOX@paraffin nanohybrid for combination of PTT and chemotherapy of HeLa cells in vitro. In their study, the laser irradiation enabled temperature-triggered fast DOX release, thus reducing the side effects of nanomedicine on normal cells.

In a recent study by Zheng and coworkers, the AuNS-based nanohybrids were designed and synthesized. In their design, AuNSs with a high photothermal conversion efficiency (50.5%) were used for PTT of tumors, while a sensitive thermo-responsive glycopolymer made up with glucose that can be further conjugated with Concanavalin A (ConA) through specific recognition and galactose with targeting specificity to asialoglycoprotein receptor on the surface of hepatocytes. Due to the targeting effect of the galactose, ConA is able to exert its therapeutic activity through causing autophagy and degradation of mitochondria to lead to cell death. Due to the exerted PTT and chemotherapy effects, the nanohybrids exhibit a significant synergistic therapeutic effect against xenografted HepG2 tumors.

Tumor resistance has always been a stumbling block in cancer treatment. To overcome this, Chen and coworkers developed the pro-apoptotic peptide TPP-KLA-functionalized AuNS-pep/DOX@HA nanohybrids for both cancer cell- and mitochondria-targeted combination PTT/chemotherapy of orthotopic SCC-7 tumors. In this design, the functionalized HA was used to direct the targeting of the nanohybrids to tumors with CD44 receptor expression, while the linked TPP units in the peptide were used for mitochondria targeting. Due to the hierarchical targeting design, significant DOX accumulation within the cells, TPP-KLA-mediated disruption of mitochondria function, and AuNS-mediated localized hyperthermia under NIR laser irradiation, efficient inhibition of the energy-dependent drug efflux pathway along with prolonged intracellular retention of DOX can be realized to overcome drug resistance for enhanced antitumor therapy.

Furthermore, to overcome the drug resistance of paclitaxel (PTX)-resistant HepG2/PTX cells, Zhu et al. functionalized PTX-loaded temperature-sensitive liposomes (PTX-TSL) onto the AuNSs, which were premodeled with a targeting ligand of 2-deoxyglucose (DG) and transmembrane peptide 9-poly-D-arginine (9R) through Au–S bonding, and siRNA through electrostatic interaction. The created PTX-TSL-siCOX-2(9R/DG-AuNS nanohybrids could exert their cooperative effects of DG targeting to cancer cells, 9R peptide-mediated transmembrane delivery of the hybrids for both PTX delivery and gene silencing of cyclooxygenase-2 (siCOX-2) involved in inducing multidrug resistance of tumors, and NIR-mediated fast release of PTX and ablation of cancer cells. These cooperative effects enabled synergistic treatment of drug-resistant HepG2/PTX cells in vitro.

### 3.3 Imaging-guided therapy

#### 3.3.1 Single modal imaging-guided PTT

Benefiting from their own composition and special structure, AuNSs have been regarded as a nanoplatform for...
imaging-guided tumor therapy. Due to the photothermal conversion effect, AuNS-based hybrids can always be used as a photothermal imaging agent. Therefore, it is important to discuss other imaging modes based on AuNSs or hybrids. In particular, the plasmon resonances of NPs strongly depend on their own composition, size, and shape.\textsuperscript{90} The LSPR of the AuNSs resulting from the hybridization of plasmons associated with the core and the branches of the particle\textsuperscript{10} can provide strong electromagnetic (EM) fields. Typically, the multiple sharp branches of AuNSs with tunable LSPR significantly amplify the EM field intensity,\textsuperscript{37} which acts as “hot spots” for SERS imaging.\textsuperscript{31} Due to the superiority of SERS imaging such as high signal-to-noise ratio, sub-picomolar level sensitivity, nonphotobleaching feature, and excellent multiplexing capability, AuNSs have been applied for multiplexed imaging of cancer.\textsuperscript{89,92,93}

CT is another most commonly used imaging technique in the clinic owing to its high spatial and density resolution, low cost, deep penetration, fast scan speed, and clear 3D tomographic features.\textsuperscript{94} Compared to conventional CT contrast agents such as iodine-based compounds (e.g., omnipaque), AuNSs containing Au with a higher atomic number than that of iodine exhibit more efficient X-ray attenuation than omnipaque,\textsuperscript{95} affording them as promising CT imaging contrast agents.\textsuperscript{96}

Further, PA imaging is a unique noninvasive bioimaging technique combining the metrics of ultrasound (US) and optical imaging. Distinct from the conventional optical imaging in the millimeter range, PA imaging has been used to realize imaging depths of 5 cm in the NIR region.\textsuperscript{97-99} Due to the LSPR at a long wavelength around 1000–1200 nm and a high absorption versus scattering coefficient, AuNSs with multiple sharp branches are regarded as favorable agents for PA imaging.\textsuperscript{21,29,100}

To realize SERS imaging-guided PTT of cancer cells, Chen and coworkers\textsuperscript{101} reported a BSA-modified AuNS-indocyanine green (ICG) nanohybrids to achieve real-time sensitive monitoring of ICG absorption within the cancer cells after PTT treatment through SERS imaging. Due to the AuNS- and ICG-mediated PTT effect, the applied SERS imaging enabled detection of temperature and concentration distribution of nanohybrids in U87 glioma cells in vitro with a subcellular spatial resolution due to the temperature-dependent property of the ICG SERS spectra.

For CT imaging-guided tumor therapy, AuNS-based nanohybrids synthesized in one pot with BSA as a template have shown a huge potential in CT imaging-guided PTT.\textsuperscript{36} In a recent study,\textsuperscript{36} we reported the development of PDA-coated AuNSs (Au-PEI@pD NSs) for CT imaging-guided PTT of tumors. The AuNSs were formed via the seed-mediated growth method, stabilized with PEI-SH via Au–S bonding, and coated with PDA. The created Au-PEI@pD NSs possessed a better photothermal conversion efficiency (49.9\%) than PDA-free AuNSs-PEI (36.1\%). At 10 min post intratumoral injection, the CT value in the tumor region dramatically increased up to a value of 424.3 HU, which was 11.3 times higher than that of before injection. Due to the fast temperature increase of the tumor region injected with the Au-PEI@pD NSs from 29.8°C to 58.7°C within 5 min under laser irradiation, the Au-PEI@pD NSs could be used as a promising nanoplatform for CT imaging-guided PTT of xenografted HeLa tumors.

Similarly, to realize PA imaging-guided PTT of tumors, AuNS-based nanohybrids synthesized in one pot with chitosan as a template have shown a huge potential in PA imaging-guided PTT of tumors.\textsuperscript{34} In another study, Huang et al.\textsuperscript{102} prepared trans-activating transcriptional activator (TAT) peptide-modified AuNSs and loaded the AuNSs within mesenchymal stem cells (MSC) extracellular microvesicles for targeted PA imaging-guided PTT of xenografted PC-3 prostate tumors through the mediation of both microvesicles and TAT peptide. Interestingly, TAT-AuNSs could be loaded into the MSC microvesicles, the lysosomes of MSC, be assembled through the attraction of van der Waals forces and the compacting pressure in the lysosomes, and then be excreted through exocytosis that could be promoted by laser irradiation. The improved outcome in PA imaging of the tumor site perfectly proved the effective targeting ability and enhanced permeability of the MSC microvesicles loaded with the TAT-AuNSs. Furthermore, to achieve deeper tissue penetration and lower scattering coefficient under an NIR-II light, Raghavan et al.\textsuperscript{103} prepared an NIR-II light-stimulated silica-coated AuNSs (DPGNS) for enhanced PA imaging-guided PTT of xenografted MDA-MA-231 tumors. Emphatically, the silica shell of the DPGNS, as a heat transfer layer, did not seem to compromise the PA imaging capability of the DPGNS. Under an NIR-II light excitation, the DPGNS showed a deep penetration of tumor tissue for PA imaging-guided PTT of tumors.

### 3.3.2 Single modal imaging-guided combination therapy of tumors

Single modal imaging-guided combination therapy can also be realized through the design of AuNS-based nanohybrids. In a recent study,\textsuperscript{20} we developed multifunctional RGD-modified dendrimer-stabilized AuNSs (AuDSNS/siRNA) nanohybrids to achieve targeted CT imaging-guided combination PTT and gene therapy of xenografted U87MG tumors overexpressing αvβ3 integrin. Through CT imaging, the penetration and distribution of RGD-Au DSNS/siRNA in tumor tissues can be monitored. Interestingly, the CT value in the tumor region at 10 min
post intratumoral injection was 28.5 times higher than that before injection. With the high siRNA transfection efficiency and obvious photothermal conversion efficiency (79%) of the RGD-Au DSNS/siRNA nanohybrids, the therapeutic effect of combination PTT and gene therapy was much more effective than that of single PTT.

In another study, Jia and coworkers developed a versatile AuNS-based nanohybrid for PA imaging-guided combination PTT/gene therapy of xenografted Capan-1 tumors. The nanohybrids were integrated with AuNSs and reduced GO, and further coated with mixed lipids of DOPE, DODAB, and DOPE-FA. With the demonstrated increased photothermal conversion efficiency from 37.9% of single AuNSs to 66.3% of the hybrids, cationic lipid-endowed powerful gene transfection capacity, FA-mediated targeting specificity to FA receptor-expressing tumors, the developed nanohybrids were able to diagnose tumors through PA imaging. Distinct PA imaging signals at the tumor site were observed at 24 h post intravenous injection of the nanohybrids. Meanwhile, compared with the PBS group, the tumor growth inhibition rate of the PTT/gene therapy group reached 98.5%, much higher than those of the groups of single-mode PTT (76.1%) and gene therapy (55.2%).

### 3.3.3 Multimodal imaging-guided PTT

Due to the defects of single modal imaging, it is essential to develop AuNS-based nanohybrids for multimodal imaging-guided PTT of tumors. In a recent work, we prepared the Fe$_3$O$_4$@Au core/shell NSs through the use of citric acid-stabilized Fe$_3$O$_4$/Ag composite NPs as seeds for AuNS growth. Owing to the decoration of PEGylated FA and respective components of AuNSs and Fe$_3$O$_4$, the created Fe$_3$O$_4$@Au core/shell NSs enabled targeted tri-mode PA/CT/MR imaging-guided PTT of a xenografted HeLa tumor model. In another work, in order to realize HA-mediated tumor targeting, we developed HA-functionalized Fe$_3$O$_4$@Au-HA NSs for targeted dual-mode CT/MR imaging-guided PTT of CD44 receptor-overexpressing HeLa tumors. Compared to preinjection, both the MR and CT contrast in the tumor region were greatly enhanced, with the T$_2$-MR signal intensity dramatically decreased by 15.6 times and the CT value increased by 14.6 times.

For SERS/MR imaging-guided PTT of xenografted MDA-MB-231 tumors, Gao et al. fabricated organosilica-coated and 3,3-diethylthiatricarbocyanine iodide (DTTC)-tagged AuNSs (MGSNs) that were further conjugated with Gd chelates. This dual-modal imaging design integrates the advantages of significant soft tissue contrast, high sensitivity, and spatial resolution. Owing to the enhanced permeability and retention (EPR) effect of leaky vasculature and poor lymphatic drainage in tumors, the MGSNs are able to effectively accumulate at tumor sites. Benefiting from the “hot spots” of the unique multiple sharp branches of the MGSNs, the enhanced Raman signal of DTTC at 507 cm$^{-1}$ could be detected at the tumor site at 30 min post intravenous injection of the MGSNs.

For other multimode imaging design, in a very recent study, we developed AuNS-decorated hollow mesoporous silica nanocapsules (HMSs) loaded with a US imaging agent of perfluorohexane (PFH) for tumor US/CT/PA imaging and PTT. Interestingly, different from the commonly adopted strategy, in which AuNSs are coated by SiO$_2$, we proposed a method to grow AuNSs on the periphery of HMSs (Figure 5). HMSs were first synthesized by selective etching, then silanized to possess thiol surface groups, and coated with Au NPs via Au–S bonding. Followed by growth of AuNSs on the HMS surface, encapsulation of PFH in their interiors, and conjugation of thiolated PEG on the particle surface, the hybrid platform was finally synthesized for precision theranostics of a xenografted C6 tumor model.

### 3.3.4 Multimodal imaging-guided combination therapy of tumors

As mentioned, a single modal imaging diagnosis may cause false-positive signal. Single modal therapy is insufficient to achieve complete tumor elimination. The emergence of AuNS-based nanohybrids that integrate multimodal imaging and combination therapy components could improve the diagnosis accuracy and tumor therapy efficiency, contributing to the clinical translation of nanomedicines.

In order to realize multimode imaging-guided combination therapy of tumors, we recently explored the development of dendrimer-modified AuNSs embedded with ultrasmall iron oxide (USIO) NPs (Figure 6). In this work, we first prepared citrate-stabilized USIO NPs and generation 5 (G5) PAMAM dendrimer-stabilized Au NPs (Au DSNPs) by a hydrothermal method and self-reduction, respectively. Then, composite Fe$_3$O$_4$/Au DSNPs with a certain Fe/Au molar ratio were obtained via covalent bonding between the amine groups of dendrimers for the Au DSNPs and carboxyl groups on the surface of USIO NPs, and were used as seeds to generate the dendrimer-stabilized Fe$_3$O$_4$/AuNSs (for short, Fe$_3$O$_4$/Au DSNFs). Through acetylation of the terminal amines of the G5 PAMAM dendrimer component, the surface potential of the Fe$_3$O$_4$/Au DSNFs was shielded to improve their biocompatibility. Benefiting from the unique structure and composition, the Fe$_3$O$_4$/Au DSNFs were endowed with a higher $r_1$ relaxivity (3.22 mM$^{-1}$ s$^{-1}$).
and photothermal conversion efficiency (82.7%) than single USIONPs and Au DSNFs, respectively. Notably, the signal intensities of MR, CT, and PA imaging simultaneously reached the maximum at 60 min post intravenous injection, demonstrating that the Fe$_3$O$_4$/Au DSNFs can be used as an excellent tri-mode MR/CT/PA imaging agent for tumor diagnosis. Likewise, under the combination of PTT/RT, the xenografted 4T1 tumors could be significantly inhibited with an efficiency much higher than single mode of PTT and RT.
In another work, Yu et al.\textsuperscript{112} designed a multifunctional AuNS-based nanohybrid to realize multimodal PA/CT imaging-guided combination PTT and chemotherapy against xenografted MDA-MB-231 tumor, a triple-negative breast cancer (TNBC, Figure 7). Iridium (Ir) complex, a promising alternative to DOX and Pt-based drugs with multiple anticancer mechanisms and strong anticancer activity and low toxicity,\textsuperscript{113} was loaded onto the AuNS surface through van der Waals interaction or hydrogen bond ($K_a = 2 \times 10^5$ M$^{-1}$). Further, polyetherimide-AE105 peptide (P-AE105), which targets urokinase-type plasminogen activator receptor overexpressed by TNBC, was also coated on the AuNS surface via strong electrostatic interaction ($K_a = 8.5 \times 10^8$ M$^{-1}$). The heat caused by NIR laser irradiation could weaken the electrostatic interaction between P-AE105 and AuNSs, and diminish the binding between AuNSs and Ir complex, thus realizing an NIR laser-triggered controlled release of Ir complex. Therefore, combining the significant photothermal performance and dual-mode PA/CT imaging property of AuNSs and the chemotherapy effect of Ir complex, the developed AuNS@Ir@P-AE105 could serve as a promising nanoplat- form for multimode TNBC theranostics.

In a very recent study by Xing and coworkers,\textsuperscript{31} HMS/AuNS core-shell nanohybrids combined with a thermosensitive gel were prepared to realize multimodal US/CT/PA imaging-guided combination PTT/chemotherapy of patient-derived xenografted AsPC-1 tumors. In this work, AuNSs grown on the surface of HMSs were used to encapsulate gemcitabine (Gem), a clinical chemotherapeutics, and PFH, a US contrast agent within the internal cavities of HMSs, and modified...
with insulin-like growth factor-1 (IGF1), a ligand for IGF1 receptor (IGF1R)-expressing cancer cells (e.g., pancreatic cancer and mesenchymal cells) through Au–S bonding on the surface of AuNSs. Through the use of thermostensitive gel, the controlled thermo-responsive release of Gem was achieved. Interestingly, the US/CT/PA imaging signal intensities at the tumor site all reached the highest value at 2 h post intravenous injection, providing versatile, complementary, and comprehensive information of a pancreatic cancer model. Combined with interventional technology, the nanohybrids enabled an efficient combination PTT and chemotherapeutic effect of a pancreatic cancer model after a single administration, ingeniously simulating the process of clinical surgical resection and postoperative chemotherapy.

4 CONCLUSIONS AND PERSPECTIVES

This review summarizes the significant progresses that have been made in the synthesis and construction of AuNS-based nanohybrids and their shaping for cancer theranostic applications. Various AuNS-based nanohybrids including metal compound/AuNSs, SiO₂/AuNSs, polymer/AuNSs, and dendrimer/AuNSs, and so forth have been created through the methods of seed-mediated growth, one pot-based seedless or template approach. The designed AuNS-based nanohybrids have been used for multimode imaging, combination therapy, as well as single mode/multimode imaging-guided PTT and combination therapy of tumors.

Certainly, based on the need for multimode imaging and combination treatment of tumors, there is still a huge room to explore the new design and theranostic application of AuNS-based nanohybrids. First, more natural products can be applied as templates and reducing agents for the synthesis of AuNS-based nanohybrids with bioactivity and biosafety. Second, most of the current developed AuNS-based nanohybrids show great PTT performance in the NIR-I (750–1000 nm) light window, leading to limited tissue penetration depth. Hence, the composition and structure can be further precisely regulated to achieve deeper penetration in the NIR-II light window. Third, the increase in oxygen content and ROS can be further facilitated by hyperthermia, so PTT-enhanced PDT combination therapy is expected to be designed with the AuNS-based nanohybrids. Lastly, PTT can cause immunogenic cell death to activate the host immunity against cancer. Therefore, the AuNS-based nanohybrids loaded with antigens, adjuvants, or immune checkpoint inhibitors may be developed for PTT-enhanced immunotherapy. It is believed that the broader and deeper exploration of the possible design and functions of AuNS-based nanohybrids than those of the state of art in the literature could promote further development of innovative therapy strategies in cancer theranostics and facilitate the clinical translation of cancer nanomedicine.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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