Review

Nano Biomed Eng

Advances and Prospects in Integrated Nano-oncology

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Abstract

In recent years, the deep integration of basic research and clinical translational research of nanotechnology and oncology has led to the emergence of a new branch, namely integrated nano-oncology. This is an emerging and important interdisciplinary field, which plays an irreplaceable role in the diagnosis, treatment, early warning, monitoring and prevention of tumors, and has become a new interdisciplinary frontier. Here main advances of integrated nano-oncology was reviewed, mainly included controlled preparation of nanomaterials, ultra-sensitive detection of tumor biomarkers, multi-functional nanoimaging probes and integrated diagnosis and treatment technology, innovative nano drugs and nano drug delivery system, DNA nanotechnology, RNA nanotechnology, nano self-assembly technology, nanosensors, intelligent nanorobots, nanotherapeutic machines. The terms, concepts, trends and challenges are also discussed with the aim of promoting the application of nanotechnology in integrated oncology and solving the scientific and key technical problems in basic and clinical translational research of cancer.

Keywords: integrated nano-oncology; nanomaterials; nanoprobes; molecular imaging; nano drug delivery system; nano diagnosis; targeted therapy

Introduction

Integrative medicine, known as holistic integrative medicine (HIM), refers to the organic integration of the most advanced theoretical knowledge in each field of medicine and the most effective practical experience in each clinical specialty from the perspective of the whole human being, and to make corrections and adjustments according to the social, environmental and psychological realities to make it a new medical system that is more in line with and more suitable for human health and disease treatment [1]. In the last decade or so, with the rapid development of nanotechnology and the deep integration of basic research and clinical translational research in nanotechnology and oncology, a new

branch, namely integrated nano-oncology, has emerged. Integrated nano-oncology, formed by combining nanotechnology and oncology, is an emerging and important intersectional field in nanobiotechnology, which plays an irreplaceable role in tumor diagnosis, treatment, prediction, monitoring and tumor prevention, and has become a new interdisciplinary frontier and one of the core areas of priority development of frontier science and technology in various countries at present. Integrated nano-oncology is a fully interdisciplinary discipline, which starts from the whole person and makes full use of the unique advantages of nanotechnology to solve the series of challenges and key scientific problems faced in the process of clinical tumor prevention, early diagnosis and treatment, carry out basic and clinical translational research, and promote the clinical translation of research results; its research scope involves all aspects of oncology, including tumor early warning, early screening, The scope of its research covers all aspects of oncology, including tumor early warning, early screening, multi-functional nanoprobes and imaging diagnosis and treatment, nano-drug delivery system, nano-tumor treatment drugs, nano-effect-based physical therapy, nano-immunotherapy, nano-treatment robot, etc., which make revolutionary changes in tumor treatment and diagnosis [2].

With the continuous emergence of new nanomaterials and the rapid development of highly biocompatible surface treatment technologies, how to apply the nano size effect, nano-surface effect, quantum effect, unique acoustic, optical, electrical, thermal and magnetic properties of nanostructures to improve the sensitivity and specificity of tumor marker detection, design good biocompatible tumor-related nanoprobes and solve theoretical problems of *in vivo* imaging in lesion localization. The design and controlled synthesis of nanodrugs and nanodrug delivery carriers and the development of novel nanotherapeutic technologies, and how nanotechnology can play a key role in tumor research are the most challenging questions posed by life sciences to nanotechnology at present, and a major national need facing the development of nanotechnology. This chapter gives a summary review of the main progresses achieved in integrated nano-oncology in the past five years, discusses the terminology, concepts and development trends, and the challenges that exist, with the aim of promoting the application of nanotechnology in integrated oncology and solving the scientific problems and key technical issues in basic and clinical translational research in oncology.

Advances in Integrated Nano-oncology

In the past five years, integrated nano-oncology has made significant progress in controlled preparation of nanomaterials, ultrasensitive detection of tumor markers, multifunctional nano-imaging probes and diagnostic and therapeutic integrated technologies, innovative nanomedicine and nano-delivery carriers, DNA nanotechnology, RNA nanotechnology, nano-self-assembly technology, nano-sensors, intelligent nano-robots, and nano-therapeutic machines, some of which have entered clinical trials/application stage, but the potential toxicity, secondary effects, metabolic pathways, and biodegradability of nanomaterials still have basic scientific problems, and new nano-bio-detection technologies based on nano-effects, high-efficiency nano-immunotherapy carriers, diagnostic and therapeutic integrated nano-imaging probes, diagnostic and therapeutic integrated nano-robots, the design and effectiveness and safety evaluation of nano-drugs, and the toxic side effects of nano-drugs. The avoidance or reduction of toxic effects of nanomedicines are important research directions in the field. The following is a review of these advances.

Types of nanoparticles for tumor treatment

So far, there are eight main types of nanoparticles used for tumor therapy, including magnetic nanoparticles, gold nanoparticles, quantum dot nanoparticles, carbon nanoparticles, silicon nanoparticles, liposome nanoparticles, albumin nanoparticles, and upconversion nanoparticles.

Magnetic nanoparticles

Common magnetic nanoparticles are mainly Fe and Co oxides, and the typical magnetic nanoparticle is Fe_3O_4. Fe_3O_4 is used as a magnetic nucleus, and then relevant functionalized modifications are made on its surface, which can be used for extraction, separation and purification of nucleic acids, and can be used as magnetic resonance imaging (MRI) probes or carry therapeutic drugs for the diagnosis and treatment of tumors. Magnetic nanoparticles are the most widely

used nanoparticles, and MRI contrast agents based on magnetic nanoparticles have been approved by FDA and used in clinical applications for many years [3, 4].

Gold nanoparticles

Research shows that: Nanoscale gold materials can effectively kill tumor cells in human body. At present, gold nanoparticles of different morphologies such as gold nanospheres, gold nanorods, gold nanoprism, gold nanoclusters, etc. are controllable and have been used in the diagnosis and treatment of tumors [5, 6].

Quantum dot nanoparticles

Quantum dot materials (QDs) are nanoscale in size in all three dimensions. With the advantages of stable photochemical properties, long fluorescence lifetime, wide excitation spectrum and narrow emission spectrum, and good biocompatibility, quantum dots have been studied more systematically in the detection of tumor markers, dynamic tracking of tumor cells and bioimaging. However, the *in vivo* application of quantum dots must address the safety issues [7, 8].

Carbon nanoparticles

Carbon nanomaterials include the following types: carbon nanotubes, carbon nanofibers, carbon nanospheres, carbon nanodots, graphene and graphene oxide. These carbon materials have been studied more systematically in nano-detection sensors, nano-drug delivery systems, etc. However, their *in vivo* metabolism and degradation still need to be further investigated, which otherwise limits their *in vivo* applications [9].

Silicon nanoparticles

SiO_2 has good biocompatibility and large specific surface area, and has been studied more systematically in drug delivery to tumors, especially mesoporous silicon has been used for drug delivery, imaging and therapy. SiO_2 has also been used for surface modification of nanomaterials and coupling with other targeting molecules [10, 11].

Liposomal nanoparticles

Self-assembly of nanoparticles and lipid vesicles in aqueous solution to form new assemblies, i.e. liposomal nanoparticles. Liposomal nanoparticles combine the advantages of liposomes and nanoparticles, which can be used as a drug carrier to achieve the treatment of tumor cells *in vivo* by combining with some antitumor drugs on its surface. Liposome formulation is the most widely used dosage form [12, 13].

Albumin nanoparticles

The most successful albumin as drug carriers are albumin paclitaxel and albumin adriamycin, which have been approved by FDA and are widely used in the clinic. Based on albumin, series of nanomedicines have been developed and some of them have entered the clinical trial stage [14, 15].

Upconversion nanoparticles

Rare-earth-doped upconversion nanomaterials are a kind of materials that produce anti-Stokes luminescence phenomenon under the excitation of infrared and near-infrared (NIR) light (usually 980 or 808 nm), and their emission frequency band is visible light band. Because of their narrow emission peaks, stable luminescence, relatively long fluorescence lifetimes and large anti-Stokes shifts, rare earth-doped upconversion nanomaterials have received widespread attention in the field of tumor therapy. Upconversion luminescent materials are usually composed of three parts: activator, sensitizer, and substrate material. The activator is used to excite the luminescent particles in the upconversion luminescence process. To avoid cross-chirality, activators are usually doped in small doses (0.5%–2%), the most commonly used activators are erbium Er^{3+} (emission peaks: 520, 540, 650 nm), holmium Ho^{3+} (emission peaks: 539 and 650 nm) and thulium Tm^{3+} (emission peaks: near infrared region near 800 nm). The sensitizer is responsible for the energy transfer of the activator to achieve luminescence efficiency. Yb^{3+} is the most commonly used sensitizer, and its corresponding excitation light is 980 nm near infrared light, usually doped at about 18%–20%. $NaYF_4$, $NaGdF_4$, $NaLuF_4$, CaF_2, $BaGd_5$, $BaLuF_5$, $BaYF_5$ and other nanocrystals are the most commonly used matrix materials, and in general, hexagonal phase matrix materials are more favorable than cubic phase matrix materials for luminescence efficiency [16, 17].

Advances in tumor marker nano-detection technology

Tumor markers (TM) are substances that change abnormally during the occurrence and proliferation of malignant tumors due to the expression of genes associated with the tumor cells or the body's response

to the tumor. Tumor markers have been found for more than 100 years. Since the 1960s, they have been widely used in clinic and played an important role in the diagnosis and treatment of tumors. With the development of biotechnology, various new markers have been discovered with increasing specificity and sensitivity, including oncogenes, tumor suppressor genes and their products, tumor DNA, cytokines and their receptors, tumor miRNAs and tumor stem cells, etc. The combined diagnosis of tumor markers has also become a hot topic of interest. The combined diagnosis of multiple markers for different tumors has greatly improved the sensitivity of tumor detection.

In the last five years, a number of significant advances have been made in the field of nano-detection. These include single gene SNP locus detection, nanopore sequencing, detection of trace breath markers and salivary markers based on SERS chip, single microRNA detection based on nanoparticle SPR effect, chromatographic chip detection technology based on magnetic nanoparticles or quantum dots labeling, multi-indicator detection technology for microfluidic chips based on giant magnetoresistance effect, magnetic impedance effect and tunneling magnetoresistance effect, quantitative microfluidic chip technology for circulating cancer cell capture combined with magnetic nanoparticle separation technology, electrochemical sensing for ultra-sensitive detection of ctDNA on the basis of nanoparticle-modified electrodes, microfluidic chip technology for exosome separation and marker detection, digital PCR chip detection technology, quantitative PCR detection technology based on nanocluster molecular beacons, combined chip technology with nanoparticle labelling, especially the series of detection technologies based on modeling combined with artificial intelligence algorithms, have significantly improved the sensitivity and accuracy of detection, and some of the technologies have entered clinical application. The main advances are as follows:

Controllable preparation of nano-plasma probes and SNP gene detection

Single nucleotide polymorphism (SNP) detection is a hot and difficult research area in DNA biosensing. Using the nanoscale addressable nature of DNA origami structures, a novel SNP amplification model of DNA nanoprobes labeling SNP sites was proposed to achieve the goal of direct reading of SNP information by atomic force microscopy (AFM) at the single molecule level (Fig. 1) [18]. The design of probes based on DNA nano origami structures allows for thousands of options, overcoming the disadvantage of having fewer types of fluorescent molecular probes to choose from in SNP direct reading techniques. The resolution of AFM is 0.2–0.3 nm compared to the 20–50 nm resolution of super-

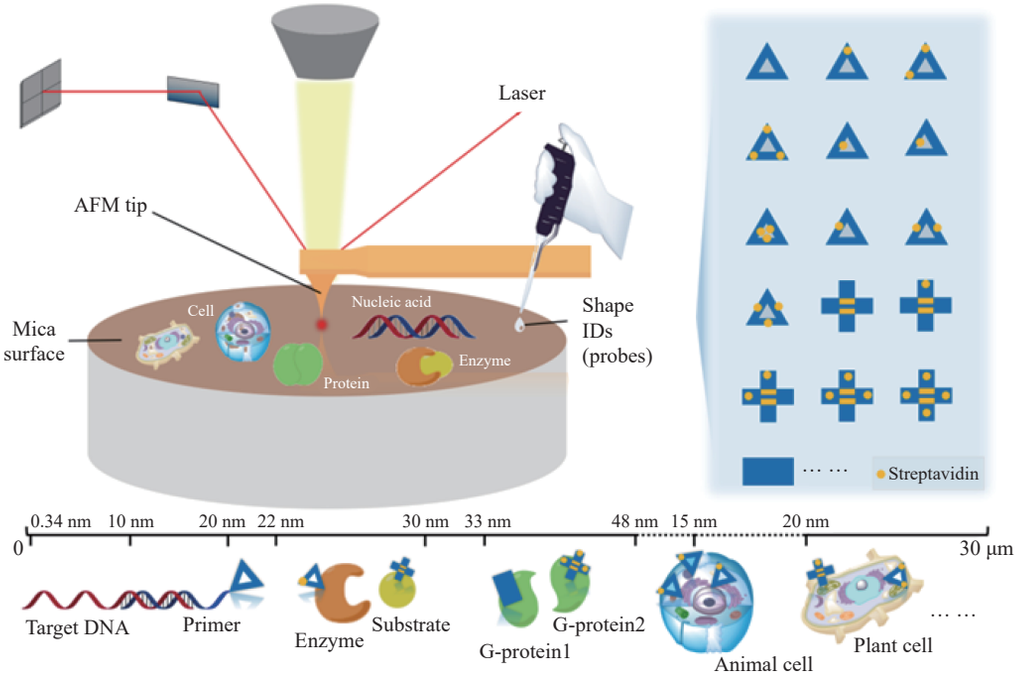

Fig. 1 Precise identification of SNPs in patient samples by AFM using DNA nanoprobes. Reproduced with permission from Ref. [18] © 2018 Springer Nature.

resolution fluorescence microscopy, which allows more information to be obtained for imaging at the single molecule level. As the images acquired by AFM have extremely precise scales, the positions of adjacent SNPs can be accurately measured, leading to accurate information on SNP positions. The method is simple, time-saving and inexpensive, and is expected to be used for the detection of high-risk groups, the identification of disease-related genes, the design and testing of drugs, and basic research in biology.

Serial detection technology based on SERS chip

(a) SERS chip for saliva marker detection

Twelve volatile organic compounds (VOC) markers were screened from patients' saliva and can be used for early gastric cancer screening. A graphene oxide monolayer was prepared, followed by in-situ synthesis of gold nanoparticles to prepare gold nanoparticle graphene oxide (Au/GO NS), which was rolled into a tubular structure under the action of ultrasound to prepare a SERS chip on the film. Saliva markers were adsorbed on the surface of the SERS chip and were detected to achieve screening of gastric cancer and differentiation of early gastric cancer and progressive gastric cancer from normal individuals with a SERS enhancement factor of 10 [8, 19]. Based on the clinical specimen detection data, a deep learning model for saliva diagnosis was developed using deep learning SVM analysis software, enabling the accuracy of saliva screening for early gastric cancer to reach 97.18%, sensitivity to 96.88% and specificity to 97.44% [20].

(b) SERS chip for nano-detection of exhaled gas markers

VOC markers were screened out to distinguish early gastric cancer, progressive gastric cancer and healthy individuals. The silver microspheres were able to enhance the SERS signal of Raman molecules up to 10^{15} times, with a detection sensitivity of 10^{-15} M [21]. Microfluidic SERS chips integrating hollow silver microspheres were developed. Combined with machine learning models, the sensitivity of detection reached 10^{-15} M, improving the accuracy of early gastric cancer screening to 97.4%.

Quantitative detection method of exosomes based on branch rolling circle amplification

A screened aptamer was used as an exosome recognition probe for gastric cancer, while the signal was amplified using branch rolling circle amplification to achieve quantitative detection of gastric cancer exosomes. An aptamer that specifically binds exosomes secreted by gastric cancer cells was screened, and a padlock probe of 63 bases in length was designed with the aptamer as the linking probe. The aptamer and gastric cancer exosomes were incubated and the unbound aptamer was removed by periplasm. The bound aptamer was eluted and treated at high temperature, and the separated aptamer was interacted with the padlock probe to form a closed-loop template. In the subsequent amplification, a second primer with the same partial sequence as the loop template was added. After hybridization with the amplified product, the primer could trigger branched chain amplification and form a length gradient double stranded nucleic acid [22]. Finally, we realized the quantitative detection of gastric cancer exosomes by using the strong fluorescence of nucleic acid dye SYBR Green Ⅰ combined with the double helix groove region of double stranded nucleic acid. The fluorescence signal of the product was positively correlated with the initial concentration of exosomes, and the sensitivity could reach 1 exosome/mL (Fig. 2) [23].

Series of detection technologies based on MoS_2 nanosheets

(a) MicroRNA detection technology based on MoS_2 nanosheets

Molybdenum disulfide (MoS_2) is a novel two-dimensional layered nanomaterial with layered structure and novel physical photoelectrochemical properties. MoS_2 is an ideal substrate material due to its large specific surface area and can be hybridized with abundant nanomaterials (such as precious metals, metal oxides) and organic molecules to form novel nanocomposites. These nanocomposites have superior optoelectronic properties, enabling highly sensitive detection of biological and chemical molecules. A simple and rapid label-free miRNA-21 detection strategy was devised using MoS_2 (MoS_2-Thi-AuNPs) nanocomposites co-modified with thionine (Thi) and gold nanoparticles. In this work, it is not only used as an electrochemical indicator, but also as a reductant of $HAuCl_4$, which is conducive to the formation of gold nanoparticles, and also provides the possibility for the realization of label free detection. The MoS_2-Thi-AuNPs nanocomposite attached to the surface of the glassy carbon electrode captures DNA forming a recognition layer that inhibits electron transfer between Thi and the electrode when the target miRNA-21 hybridizes with the probe DNA,

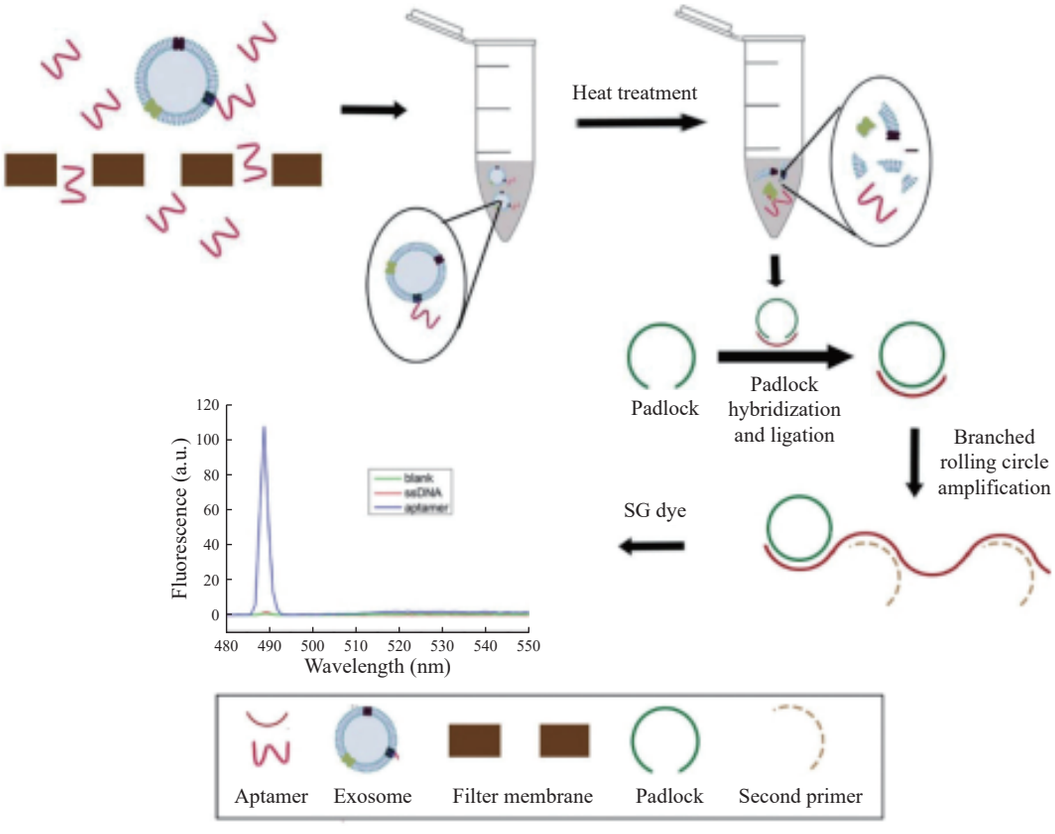

Fig. 2 Schematic illustration of gastric cancer exosomes detection based on branched rolling circle amplification. Reproduced with permission from Ref. [23] © 2020 The Royal Society of Chemistry.

resulting in a reduction in signal. Highly sensitive detection of the tumor nucleic acid marker miRNA-21 by measuring the Thi electrochemical signal, with a linear range of 1.0 pmol/L–10 nmol/L and a detection limit of 0.26 pmol/L. The MoS_2-Au@Pt nanocomposite was also prepared by functionalizing gold-platinum core–shell bimetallic nanoparticles onto a monolayer of molybdenum disulphide, enabling highly sensitive enzyme-free electrochemical detection of biomolecules using the material's good catalytic oxidation properties [24].

(b) Construction of double block DNA AuNPs probe based on MoS_2 for detection of ctDNA

MoS_2 nanosheets were prepared by lithium ion insertion and stripping method. The double-block DNA-AuNPs probes were prepared by ageing with salt. Recognition sequences with different lengths of polyA (A5capture, A10capture, A20capture, A30capture) were mixed with AuNPs in the same molar ratio of 200:1 and gently shaken overnight at 25 °C at room temperature. Subsequently, 1 mol/L phosphate buffer saline (PBS) (100 mmol/L PB, 1 mol/L NaCl, pH 7.4) was added at half-hourly intervals, in five additions, to give a final concentration of 0.1 mol/L PBS (10 mmol/L PB, 0.1 mol/L NaCl, pH 7.4). After 36 h of gentle shaking, the excess DNA was removed by centrifugation (12 000 r/mim, 20 min, 10 °C) and washed three times with 0.1 mol/L PBS solution. The obtained polyA-mediated DNA-AuNP was dispersed in a stock solution (0.1 mol/L PBS, pH 7.4). After hybridization of 1 μmol/L PIK3CA ctDNA with 20 μL of 10 nmol/L double-block DNA-AuNPs probe at room temperature for 10 min, Exo III was added to react for 30 min with a final concentration of 0.1 U/μL of Exo III. The probe was then diluted to 100 μL with 0.1 M PBS, MoS_2 was added to the mixture to give a final concentration of 10 μg/mL and incubated for 90 min before ultraviolet (UV)–visible (Vis) characterisation and recording the change in absorbance. Individual PIK3CA ctDNA detection was achieved [25].

Ag NC/DNA probes for in situ detection of microRNA biomarkers in tissue sections

Developed Ag NC/DNA probes as molecular beacons, GC-enriched hairpin-captured probes, successfully applied in FISH hybridization of gastric cancer tissues, enabling the identification of individual microRNA biomarkers within pathological

tissues, which can be used for gastric cancer diagnosis [26].

Intelligent plasmonic nanobiosensor for single-molecule microRNA detection

The gold–silver core–shell nanocubes (Au–Ag NCs) with localized surface plasmon resonance (LSPR) have emerged as promising biosensors due to their excellent chemical stability, sensitivity, label-free detection, and real-time analysis capabilities. In comparison to single-stranded DNA (one-dimensional (1D)) and hairpin DNA (2D) probes, the 3D nanostructured DNA probes exhibit enhanced spatial positioning and target capture capabilities. In this study, we developed tetrahedral probe DNA-modified Au–Ag NCs, taking advantage of the precise control over the size, vertex position, and introduction of functional sequences offered by the tetrahedral nanostructure DNA (tsDNA). This enabled the fabrication of tsDNA-functionalized single-particle LSPR optical probes, forming the basis of an "intelligent" plasmonic nanobiosensor. Not only could we achieve the detection of gastric cancer biomarker miRNA-21 at the single-molecule level (detection limit: 1 amol/L; detection dynamic range: 1 amol/L–1 nmol/L), but we also successfully demonstrated DNA-based logic computing and biological memory capabilities [27]. This research holds great promise for sensitive analysis of biomolecules and clinical diagnostics, offering broad applications in various fields.

High-bridge microfluidic chip for capture and separation of circulating tumor cells (CTCs)

By combining magnetic bead-based capture of cancer cells, we have developed a high-bridge microfluidic chip for the capture and separation of circulating tumor cells (CTCs). The chip consists of upper and lower channels, where immunomagnetic beads are employed for the recognition of CTCs. The sample is introduced into the lower channel of the microfluidic chip and, upon passing through the magnetic field region, the CTCs captured by the magnetic beads are separated and directed into the upper channel. With the utilization of this chip, we have achieved the removal of red blood cells and white blood cells, as well as the recovery and quantification of gastric cancer cells, with a sensitivity of 2 CTCs per 3 mL of blood and a specificity exceeding 98%.

Gastric cancer cells were captured using antibodies specific to the gastric secretin receptor, a gastric cancer-specific biomarker. Once captured, the cells were attracted by the magnetic chip and transported to the upper layer of microchannels through a high-bridge configuration. Meanwhile, white blood cells and red blood cells remained in the lower layer of microchannels, enabling efficient separation with a high survival rate. The separation efficiency reached 93.2%, and the cell viability was 97.5%. The flow rate used was 20 μL/min (10^6 cells/min) [28].

Integrated microfluidic chip for single-cell sorting and analysis of CTCs

We have developed a microfluidic platform that enables the integrated sorting and analysis of CTCs at the single-cell level. This chip combines size-based filtration and immunofluorescence identification methods, employing a capture-and-release strategy for the individual sorting of CTCs, thus significantly enhancing the purity of the CTCs population. Additionally, an integrated analysis module has been incorporated at the back end of the chip to enable epithelial-mesenchymal transition analysis of the target cells. By integrating cell sorting and analysis on a single chip, we have minimized cell loss, simplified experimental procedures, and improved detection efficiency. The chip achieves capture and release rates of CTCs exceeding 97% and demonstrates recovery and sorting purities both above 92% [29].

Microfluidic chip for molecular subtyping in guiding targeted therapy for gastric cancer

Integration of giant magnetoresistance (GMR) effect of magnetic nanoparticles, isothermal amplification and nucleic acid hybridization detection, biomimetic micro-mixers, and microfluidic chip technology has been achieved to develop a portable microfluidic GMR sensing system [30]. This system enables accurate molecular subtyping diagnosis within 20 minutes and facilitates rapid genetic profiling of Her2, K-ras, VEGF, EGFR, PIK3CA, and PD-1/PD-L1 mutations through combined detection.

Nanoparticle-labeled multiplex chip detection technology

Proposal of a novel principle and method for nanoparticle-labeled multiplex chip, addressing the challenge of flexible combination detection in multi-analyte testing. Based on this, a chip for *Helicobacter*

pylori typing detection was developed. H. pylori is a primary risk factor for gastric cancer, and establishing a typing diagnostic method for H. pylori is an important approach for early gastric cancer prevention [31]. Leveraging the principle of the multiplex chip, in collaboration with a partner company, we cloned and expressed CagA, Vac A, and urease of H. pylori, and prepared a chip with detection sites for CagA, Vac A, and urease. Gold nanoparticle clusters were utilized for labeling CagA, Vac A, and urease antibodies, and a fluorescence-based quantitative protein chip detection method for CagA, Vac A, and urease was established. A chip reader was developed, and cut-off values and detection standards were established. Enterprise standards were also established. The developed detection chip and reader obtained medical device Class 3 and Class 2 certificates, respectively.

Microfluidics chip for detection of multiple blood markers

A GMI microfluidics chip for simultaneous detection of CEA, CA19-9, CA125, VEGF, CA72-4, Gastrin 17, PGI and PGII was developed, 500 clinical blood samples were collected and tested, which can effectively screen out gastric cancer patients, with a coincidence rate of more than 90%. It has clinical conversion value. On this basis, a handheld fluorescence spectrometer has been developed for the rapid detection of CEA, a blood marker for gastric cancer. It can scan and quantitatively analyze the results of chromatography chip detection, and the detection results can be uploaded to databases and mobile phones [32].

Development of a detection system based on smartphones

We have developed a chromatography chip for rapid detection of gastric cancer biomarkers CA72-4 and CEA, and a software system for quantitative analysis of gastric cancer marker detection results based on smartphones [33]. We have achieved quantitative analysis of detection results and automatically uploaded data to cloud management systems and cloud databases, facilitating analysis of home and field detection results. The software developed obtains software copyright.

Magnetic nano immunochromatographic detection chip combined with artificial intelligence algorithm

We have developed a magnetic nanoparticle labeled CA724 immunochromatographic detection chip for gastrointestinal tumor markers, which qualitatively detects 1 IU/mL and quantitatively detects 0.38 IU/mL (with a clinical positive threshold of 6 IU/mL).

We have developed testing equipment for matching magnetic chromatography chips to achieve rapid and quantitative detection of single or multiple indicators of the chromatography chip, with an accuracy of 10^{-7}–10^{-4} oe. This part of the work has developed an ultra sensitive detection platform for magnetic immunochromatography chips, which can achieve fast and accurate detection of weak magnetic signals on the chromatography chip. The entire platform consists of three parts: device detection terminal, immune chromatography chip, and data server. The detection terminal is the main part of the entire platform, consisting of ultra sensitive magnetic sensors, mechanical transmission devices, and digital signal circuits, used to extract, amplify, and transmit chip signals. The immunochromatographic chip uses magnetic nanoparticles as probe markers for the detection of different diseases, including CA724, CA199, CEA, cTnI, CKMB, and Myo. The data server is the data processing part of the entire system, which can store, query, optimize, and share sample information remotely. In terms of data signal processing, machine learning methods have been adopted to achieve accurate classification processing for signals from low concentration chips, greatly improving the detection rate and accuracy of weak signal chips. Finally, through clinical samples, the platform was verified to have high sensitivity and specificity, good consistency with clinical testing, and high clinical application value [34].

Development of a small liquid phase biochip detector and detection of multiple tumor indicators

In order to transform the new liquid phase biochip detection technology based on quantum dot fluorescence encoded microspheres into the market and clinical practice, we have collaborated with well-known *in vitro* diagnostic enterprises in China to successfully develop a small liquid phase biochip detection instrument based on the optical characteristics of quantum dot fluorescence encoded microspheres, And it has been promoted and promoted at well-known domestic and foreign professional medical device exhibitions such as the 50th International Hospital and Medical Equipment

Exhibition in Dusseldorf, Germany (MEDICA2018) and the 81st China International Medical Device (Spring) Expo in 2019. Relevant detectors are preparing to apply for medical device registration certificates to achieve clinical conversion and market application of new liquid phase biochip technology as soon as possible.

A new liquid phase chip multi indicator detection system based on aggregation induced luminescence (AIE) material microspheres and nanospheres was constructed: AIE molecules with different luminescent properties were encapsulated into the polymer matrix using SPG membrane emulsification method, and AIE microspheres and nanospheres with uniform and adjustable particle size, excellent and adjustable luminescence performance, and good stability were obtained. 30 kinds of fluorescent codes were successfully obtained by using single wavelength coding method. Further, a new liquid chip detection system was successfully constructed with AIE micro sphere as coding microsphere and AIE nano sphere as fluorescence reporting molecule, combined with flow cytometry, and five kinds of allergen antibodies were simultaneously detected. Due to the excellent fluorescence amplification effect of AIE nanospheres, the detection sensitivity of this system is 2–5 times higher than that of liquid chip detection systems using commercial fluorescent dyes as reporting molecules. The results of its application to the detection of serum samples from allergen patients showed that its detection performance was comparable to that of the ImmunoCAP method, further proving the effectiveness of the detection system and laying a solid foundation for its further application to the multi indicator detection of tumor markers [35].

Advances in integrated nanoprobes for tumor diagnosis and treatment

The concept of tumor diagnosis and treatment integration was first proposed by Harrell and Kopelman in 2000 [36]. Tumor diagnosis and treatment integration requires the integration of imaging diagnosis and therapeutic functions, enabling the dual functionality of imaging diagnosis and treatment under the same time period and injection dosage conditions. Multifunctional nanoprobes are the premise and foundation for achieving tumor diagnosis and treatment integration, mainly including specific identification of key target molecules, composition of nanoparticles, and therapeutic functional drugs. In the past five years, with the rapid development of multifunctional nanoprobes technology, nanodiagnosis and therapy integration techniques have shifted towards practical clinical applications.

Molecular imaging (MI) is the science of imaging specific molecules at the tissue, cellular, and subcellular levels, reflecting changes at the molecular level in the living body, and the qualitative and quantitative study of their biological behavior in imaging, a concept first introduced by Professor Weissleder of Harvard University in 1999 [37]. Common imaging techniques in molecular imaging include: (1) nuclear medicine imaging, (2) magnetic resonance imaging, (3) optical imaging: including diffusion optics imaging, multiphoton imaging, *in vivo* microscopy imaging, near-infrared fluorescence imaging and surface confocal imaging, etc. (4) ultrasound imaging: mainly using microbubble contrast-mediated to detect changes at the cellular and molecular levels in the early stages of disease. Over the past decade, a series of efficient and innovative diagnostic and therapeutic integrated multifunctional molecular imaging probes have been developed, which have realized simultaneous diagnosis and treatment, and simultaneous monitoring of treatment, providing new means for targeted therapy at the molecular level of tumors.

Diagnostic and therapeutic integrated nanoprobes based on magnetic nanoparticles

Wang et al. first demonstrated high expression of α subunit of ATP synthase in 94.7% of gastric cancer tissues [38]. They prepared a humanized monoclonal antibody, HAI-178, and developed HAI-178 antibody-conjugated fluorescence-magnetic nanoparticles probes, achieving targeted dual-modal imaging and magnetothermal therapy of gastric cancer. The study proved that HAI-178 antibody possesses therapeutic functions for gastric cancer.

Yin et al. first designed flower-shaped Fe_3O_4@Au-HPG-Glc nanoprobes that target gastric cancer tissues [39]. By applying an external magnetic field, the rotation of nanoflower particles in gastric cancer tissues was achieved, effectively killing gastric cancer cells. The concept of a magnetic scalpel was proposed, allowing simultaneous implementation of

magnetic resonance imaging and magnetothermal therapy, resulting in enhanced therapeutic effects.

Pan et al. reported that the fifth-generation dendrimer-modified magnetic nanoparticles can carry a large number of antisense nucleic acids (Fig. 3), efficiently entering gastric cancer cells, and releasing the antisense nucleic acids within the cells [40]. The released antisense nucleic acids bind to mRNA, inhibiting the translation of target genes into proteins, ultimately suppressing tumor cell growth. The dendrimer-modified magnetic nanoparticles also prevent rapid degradation of the antisense nucleic acids by endogenous enzymes in cells, prolonging their action time and enhancing the therapeutic efficacy of antisense nucleic acids. By utilizing the characteristics of magnetic nanoparticles and combining them with an external magnetic field, magnetothermal therapy and nuclear magnetic resonance imaging of cells can be achieved. By modifying dendrimers with folic acid or RGD, targeted delivery and synchronous intracellular imaging of antisense nucleic acids, siRNA, etc., can be achieved. Regarding the mechanism of efficient entry of dendrimer-modified magnetic nanoparticles into tumor cells, it was found that dendrimers can induce the formation of transient nanoscale pores on the cell surface, allowing rapid entry of nanoprobes into tumor cells. A hypothesis on a novel mechanism of nanoprobe entry into cells was proposed. This mechanism was also confirmed by theoretical calculations, suggesting that dendrimers, through interaction between surface positive charges and the negative charge on the cell membrane surface, can instantly induce the formation of nanoscale pores on the cell surface, which quickly close again. This provides a theoretical basis for elucidating targeted imaging and synchronous therapy of therapeutic drugs for gastric cancer.

Diagnostic and therapeutic integrated nanoprobes based on quantum dots

RNAse A has high temperature resistance and anti-tumor activity. Using its properties, RNAse A-assisted synthesis of CdTe quantum dots was prepared, and then conjugated them with RGD, resulting in the formation of RGD-conjugated RNAse A-QDs probes [41]. The prepared quantum dot targeted nanoprobes exhibit good biocompatibility and actively target gastric cancer cells *in vivo*. They enter the cytoplasm of gastric cancer cells and release RNAse A enzyme, which disrupts mRNA and inhibits

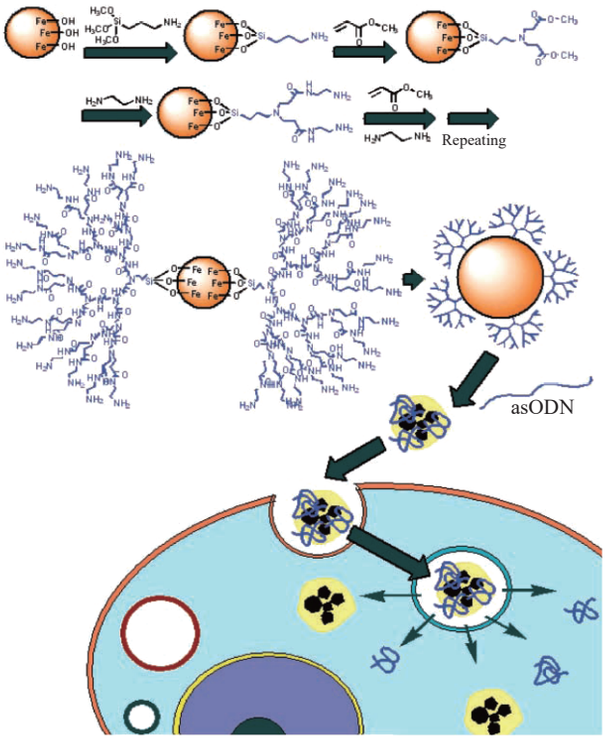

Fig. 3 Growth of pamam dendrimer on the surface of magnetic nanoparticles for nonviral gene transfection. Reproduced with permission from Ref. [40] © 2007 American Association for Cancer Research.

mRNA translation into proteins, significantly suppressing the growth of gastric cancer cells.

Based on this, HER2 antibody-conjugated RNase-QDs nanoprobes were prepared [42]. In addition, *in vivo* tumor tissue was utilized to establish an *in situ* gastric cancer model. Ultimately, targeted fluorescence imaging of in situ gastric cancer with a diameter of 3 mm was achieved, along with the inhibition of gastric cancer cell growth and an extended survival time in tumor-bearing mice. This probe consists of quantum dots with a diameter of 2.7 nm, which exhibit rapid *in vivo* distribution, excellent targeting ability, and renal excretion without significant toxicity to internal organs, demonstrating good safety.

BRCAA1 (AF208045) is a new tumor-related gene discovered in 1999, located at 1q42.1–q43, belonging to the ARID4B family. The corresponding monoclonal antibody of this protein was prepared. Immunohistochemical staining experiments on more than 200 cases of gastric cancer tissue specimens showed that this protein was highly expressed in 65% of gastric cancer tissues. Nanoprobes formed by linking this monoclonal antibody with fluorescent magnetic nanoparticles, can actively target

subcutaneous gastric cancer with a diameter of 5 mm in nude mice, and realize targeted fluorescence imaging and nuclear magnetic resonance imaging of gastric cancer. Under the external magnetic field, the local treatment of gastric cancer is realized, which has the prospect of clinical transformation.

Li et al. designed and synthesized a novel amphiphilic polymer composed of zigzag alkyl chains and multiple carboxyl chains [43]. The synthesized amphiphilic polymer was used to modify the core-shell structure of CdSe/ZnS QDs. The results showed that the fluorescence signal of the modified QDs was strong and exhibited no quenching phenomenon. They were highly stable within a pH range of 5–13 and showed no cytotoxicity towards cells. Subsequently, the QDs were respectively conjugated with BRCAA1 antibody (red QDs) and Her2 antibody (green QDs). The prepared QD probes actively targeted gastric cancer cells *in vitro*, with Her2 antibody-conjugated QDs binding to the surface of gastric cancer cells and BRCAA1 antibody-conjugated QDs located in the cytoplasm of gastric cancer cells. Electron microscopy also confirmed that the prepared QD probes could be engulfed by gastric cancer cells, allowing for the labeling of individual gastric cancer cells. Furthermore, a nude mouse model of gastric cancer was established, and after intravenous injection of the QD probes, they actively targeted the gastric cancer cells *in vivo*. Cell counting analysis of fluorescence imaging showed strong fluorescence signals with only 4200 cells. The fluorescence signal of the prepared QD targeted probes remained stable without any quenching for three months, indicating their high stability. Therefore, the prepared gastric cancer targeted QD probes hold promise for clinical translation in terms of cancer cell labeling and targeted imaging.

Diagnostic and therapeutic integrated nanoprobes based on nanocarbon dots

Carbon dots are zero-dimensional semiconductor nanocrystals with an approximate spherical shape and a diameter smaller than 10 nm. They are nanoclusters composed of a small number of molecules or atoms. Carbon dots typically have a diameter of only a few nanometers and a molecular weight ranging from a few thousand to several tens of thousands. They are generally composed of four basic elements: C, H, O, and N. The wavelength of carbon dots can be increased through modification. The luminescent properties of carbon dots are mainly manifested in photoluminescence and electrochemiluminescence, with fluorescence being the most prominent feature. As a nanomaterial with the potential to play an important role in various fields, carbon dots exhibit excellent fluorescence properties, including broad and continuous excitation spectra, single-wavelength excitation, and multi-wavelength emission. They also possess high fluorescence stability and resistance to photobleaching. The fluorescence wavelength of carbon dots can be tuned, and some carbon dots exhibit upconversion fluorescence properties. Furthermore, carbon dots act as excellent electron donors and acceptors and exhibit light-induced electron transfer characteristics. Of particular importance is their excellent water solubility, scalability in production, low cost, absence of heavy metals, environmental friendliness, and safety in use.

Huang et al. synthesized carbon dots using a nitric acid oxidation method and conducted a systematic safety evaluation of the carbon dots [44]. The results showed that the synthesized carbon dots exhibited no acute toxicity, subacute toxicity, or genotoxicity and displayed strong fluorescence signals. When coupled with the photosensitizer ce6, the fluorescence signal of carbon dots was found to be transferred to ce6 through the Förster resonance energy transfer (FRET) process, significantly enhancing the fluorescence signal of ce6 and its photodynamic therapy function. Due to the existence of the enhanced permeability and retention (EPR) effect, carbon dot probes conjugated with ce6 were observed to accumulate in a mouse gastric cancer model (Fig. 4), demonstrating enhanced fluorescence imaging and photodynamic therapy effects, leading to a significant inhibition of gastric cancer growth in nude mice. The ce6-conjugated carbon dots exhibited the following advantages: enhanced water solubility and stability of the complex, prolonged *in vivo* circulation time of ce6, improved ability to penetrate membrane barriers and tumor vasculature, and enhanced accumulation at the tumor site. The carbon dots indirectly excited ce6, enabling ce6 to exert its therapeutic function. Considering the good biocompatibility of carbon dots and the clinical application of ce6, the ce6-conjugated carbon dots hold broad prospects for clinical imaging diagnosis and simultaneous treatment of gastric cancer.

Using tryptophan as a soft template, the auxiliary synthesis of carbon dots has a significantly enhanced fluorescence signal; PEI modification of its surface

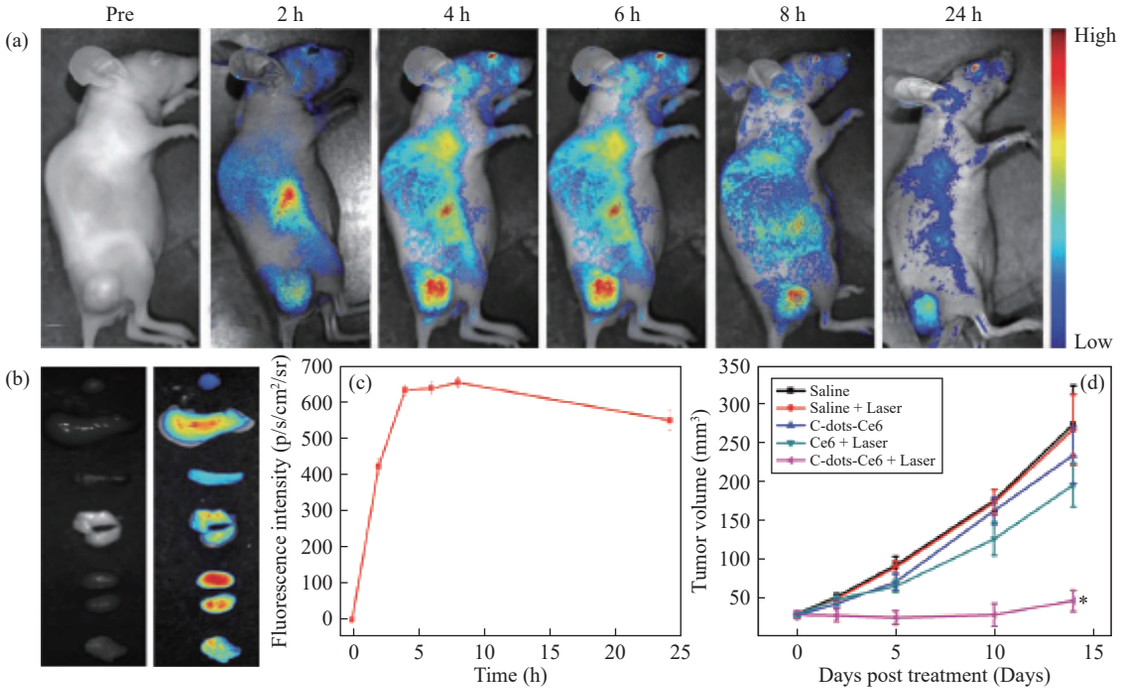

Fig. 4 *In vivo* distribution and therapeutic effects of Ce6-conjugated carbon dot probes in a nude mouse gastric cancer model Reproduced with permission from Ref. [44] © 2012 John Wiley & Sons.

can be used for targeted imaging and treatment of gastric cancer with accompanying siRNA.

An RNA enzyme-assisted synthesis of carbon dots was established to increase the quantum yield of carbon dots by 24%, demonstrating that carbon dots can enter the nucleus of tumor cells and are a good delivery vehicle. Using honey as raw material, the nuclei of the rows were controlled by microwave thermal cleavage to generate highly uniform sized carbon nanoprobes with efficient near-infrared *in vivo* imaging properties, which were used for photoacoustic imaging to improve the photoacoustic contrast signal of lymph nodes by 51 times.

Diagnostic and therapeutic integrated nanoprobes based on gold nanoparticles

RGD-coupled dendrimer-modified gold nanorod probes: gold nanorods have two absorption peaks, one at 520 nm and the other at around 820 nm. The second absorption peak is in the near-infrared region, which can absorb near-infrared light to produce thermal effects, and can be used for both fluorescence imaging and thermal imaging of tumors, as well as photothermal therapy of tumors, which has broad clinical application prospects. However, gold nanorods are often synthesized with the toxic surfactant cetyltrimethylammonium bromide (CTAB), and how to eliminate the toxicity of CTAB is a problem that must be solved before gold nanorods can be used in biomedical applications. Li et al. successfully prepared gold nanorods modified with tree molecules by using tree molecules to replace CTAB molecules on the surface of gold nanorods, and then connected with RGD peptide to prepare a nanoprobe that can actively target tumor vessels *in vivo* and kill tumor cells and significantly inhibit tumor growth under near-infrared light irradiation [45]. Animal experiments showed that this probe can make part of the local tumor tissue disappear.

Folic acid-conjugated gold nanorod probes: Huang et al. developed folic acid-linked silicon-modified gold nanorod probes [46]. Animal experiments demonstrated that the prepared nanoprobes could target gastric cancer tissues *in vivo*, enabling near-infrared fluorescence imaging of subcutaneous gastric cancer tissue with a diameter of 5mm, as well as computed tomography (CT) imaging and localized photothermal therapy. A particularly significant finding was that the gold nanorods, upon entering tumor cells, could significantly enhance the sensitivity of tumor cells to radiotherapy, reducing the dosage by 20%–35%. This phenomenon holds broad prospects for clinical application in tumor radiotherapy.

Building upon this, Chen et al. prepared silver nanoparticle-modified gold nanorods, which were conjugated with VEGF antibodies [47]. These

nanorods could target the blood vessels of gastric cancer, achieving both photoacoustic imaging and photothermal therapy of gastric cancer tissues. Additionally, they enabled highly sensitive detection of gastric cancer tissues using surface-enhanced Raman spectroscopy (SERS) and tumor boundary localization (Fig. 5).

PEG-modified gold nanoprism probes for digestive tract tumor photoacoustic imaging: The identification and targeting of digestive tract tumors pose a challenging problem due to the empty intestinal lumen. Ultrasound imaging holds significant practical significance for deep tumor tissue examination, but its diagnostic capabilities primarily rely on empirical methods. If targeted imaging of deep tumor tissues could be achieved, along with enhanced detection signals, precise diagnosis of clinical deep tissue tumors could be realized. Bao et al. designed and prepared gold nanoprism probes with PEG surface modification [48]. Cellular experiments demonstrated that PEG-modified gold nanoprism probes could actively enter tumor cells. Animal experiments showed that the prepared gold nanoprism probes could enter tumor tissues *in vivo* and significantly enhance the photoacoustic imaging signal of gastric cancer cells under laser excitation. In comparison to gold nanorods, the enhanced signal intensity of gold nanoprism probes was significantly higher, offering significant advantages in photoacoustic imaging. Moreover, these probes could generate heat and be used for synchronous tumor therapy.

Gold nanostar probes for targeted photoacoustic imaging and photothermal therapy of gastric cancer stem cells: Extensive research has indicated that gastric cancer stem cells form the basis for gastric cancer recurrence and metastasis. Previous studies revealed that gastric cancer stem cells express molecular markers such as CD24, CD44, CD133, CD166, and EpCAM on their surfaces. Successful separation of gastric cancer stem cells was achieved using CD44 antibodies, and a gastric cancer mouse model was developed using the sorted gastric cancer stem cells. To achieve targeted imaging and synchronous therapy of gastric cancer stem cells *in vivo*, gold nanostars were designed and synthesized, followed by surface modification with PEG and conjugation with CD44-V6 antibodies [49]. The results demonstrated that CD44-V6 antibody-conjugated gold nanostars could actively target gastric cancer stem cells *in vitro* and be engulfed by these cells, exhibiting strong fluorescence signals. A gastric cancer stem cell mouse model was also established, and after intravenous injection of the prepared nanoprobes for 2 hours, the nanoprobes accumulated in the blood vessels of gastric cancer tissues. Clear photoacoustic imaging of gastric cancer blood vessels was obtained using a photoacoustic imaging device, and thermal imaging revealed a temperature of approximately 65.4 °C at the tumor site, significantly disrupting local tumor tissues and enabling localized photothermal therapy. In comparison to the control group, the prepared nanoprobes could be used for targeted imaging and synchronous photothermal therapy of gastric cancer stem cells, significantly inhibiting tumor growth and holding great promise for clinical translation.

Integrated gold nanostar probes for *Helicobacter pylori* diagnosis and treatment: Approximately 75% of gastric cancer cases worldwide can be attributed to inflammation and damage caused by *H. pylori*, which the World Health Organization has classified as a Group 1 carcinogen. Preventing gastric cancer requires addressing the

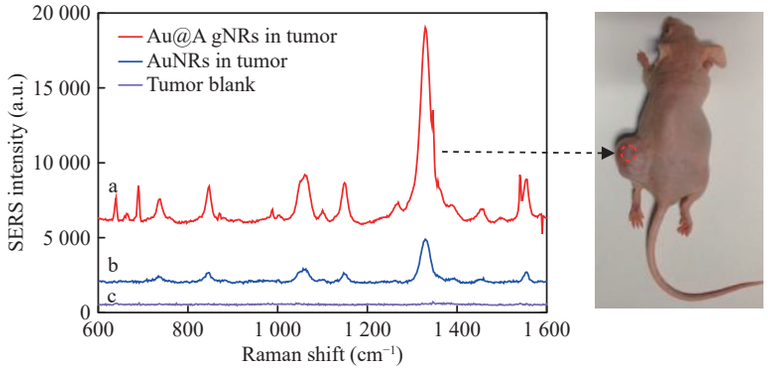

Fig. 5 Raman spectroscopic detection of subcutaneous gastric cancer targeted by silver-modified gold nanorods. Reproduced with permission from Ref. [47] © 2016 Chen et al.

issues of *H. pylori* typing, diagnosis, and treatment. pH-sensitive GNS@Ab nanoprobes for targeted imaging and photothermal therapy of Helicobacter pylori were developed [50]. Sequencing analysis demonstrated that these probes did not affect the intestinal microbiota, were not absorbed by the gastrointestinal tract, and were excreted from the body within 7 days. Clinical translation studies are currently underway.

Gold nanocluster probes for fluorescence imaging and photodynamic therapy: Gold nanoclusters (AuNCs) consist of a few to tens of gold atoms and have a size smaller than 2 nm, exhibiting sub-nanometer structures. This unique structure gives gold nanoclusters distinct physicochemical properties that differ significantly from larger gold nanorods and nanoparticles. Similar to quantum dots, gold nanoclusters exhibit quantum confinement effects. However, due to their size approaching the Fermi wavelength, the Fermi level splitting into independent energy levels prevents gold nanoclusters from supporting the quantum plasmon effect. Energy band splitting near the Fermi level leads to the formation of independent energy levels, resulting in molecular-like optical properties for AuNCs. The electron transitions between different energy levels generate fluorescence. Gold nanoclusters possess physicochemical characteristics that surpass traditional organic dyes, including strong fluorescence signals, excellent fluorescence stability, and high resistance to photobleaching. Consequently, gold nanoclusters with their favorable physicochemical properties and biocompatibility have found wide applications in the field of biological labeling.

To explore the potential of gold nanoclusters for dual-mode imaging applications, Zhang et al. prepared silica-coated core–shell gold nanoclusters (AuNCs@SiO_2), which retained the fluorescence properties of gold nanoclusters [51]. The core-shell structure protected the gold nanoclusters from interference and influences of the external complex environment, thereby stabilizing their physicochemical properties. Folic acid was conjugated with AuNCs@SiO_2 for fluorescence imaging of gastric cancer cells and dual-mode imaging of a gastric cancer animal model. The experimental results demonstrated that the prepared AuNCs@SiO_2 exhibited excellent photostability, easy surface modification and functionalization, and maintained the favorable fluorescence properties of AuNCs. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay results showed that AuNCs@SiO_2-FA had low cytotoxicity to gastric cancer cells and normal cells at high concentrations, indicating its safety, efficacy, and good biocompatibility, making it suitable for widespread application in nanobiological detection. A comparison and analysis of three sets of cellular fluorescence imaging experiments revealed that the prepared bioprobe AuNCs@SiO_2-FA could specifically target gastric cancer cells for fluorescence imaging. Additionally, CT imaging results showed that AuNCs@SiO_2 exhibited a clear CT signal in a gastric cancer tumor model in nude mice. Furthermore, a nanosensor was developed by coupling gold nanoclusters with the photosensitizer Ce6, achieving dual-modal fluorescence and CT imaging of gastric cancer and photodynamic therapy.

In their research, Zhang et al. discovered that chiral gold nanoclusters exhibited GSTP1-dependent cytotoxic effects [52]. D-GSH-AuNCs could inhibit the growth of gastric cancer cells only when the cancer cell lines had GSTP1 methylation. This result suggests that special gold nanocluster structures have selective therapeutic functions against cancer cells.

Zheng's research group at the University of Texas at Dallas used radioactive isotopes of gold, such as 198Au, during the synthesis of gold nanoclusters [53]. By incorporating 198Au into small-sized gold nanoparticles (approximately 2.6 nm) during the synthesis process, they created probes with dual-modal imaging capabilities, including near-infrared fluorescence imaging and single-photon emission computed tomography (SPECT) imaging. By utilizing these two imaging modalities, the researchers could gain a more comprehensive understanding of the nanoparticle's pharmacokinetics and behavior similar to small-molecule drugs (metabolism). The SPECT signal was observed strongly in the kidney area 10 minutes after intravenous injection, and intense fluorescence and SPECT signals were observed in the bladder area after 1 hour. At 24 hours, fluorescence and SPECT signals were no longer detectable in the mice, indicating short retention time in the body. The rapid renal and urinary excretion of the gold nanoparticles significantly reduced the chances of nonspecific accumulation and *in vivo* toxicity, thus demonstrating the potential of further developing a

nanoplatform that carries drugs and possesses therapeutic functions.

Integrated nanoprobes for diagnosis and treatment based on upconversion nanoparticles

On the basis of upconversion nanoparticles, rare-earth fluoride nanocrystals can be excited by 980 nm near-infrared light and emit light in the wavelength range of 400–800 nm. Due to their narrow emission peaks, long fluorescence lifetimes, photostability, and low toxicity, upconversion nanocrystals have great potential in biomedical engineering. We established a two-phase method involving oil and ionic phases to synthesize upconversion rare-earth fluoride nanocrystals, with controlled particle diameters below 25 nm [54]. Ma et al. coupled folate with upconversion nanocrystals and successfully achieved CT, fluorescence, and magnetic resonance imaging of subcutaneous gastric cancer models [55]. They developed a folate-conjugated upconversion nanocrystal probe for triple-modal imaging (NIR fluorescence, CT, and MRI) of gastric cancer, enabling targeted drug delivery and treatment. Safety evaluations of the upconversion nanocrystals demonstrated their safety within the dosage range for a one-time diagnosis.

Integrated nanoprobes for diagnosis and treatment based on other inorganic nanoparticles

Inorganic nanoprobes based on other types of nanoparticles have also been developed for integrated diagnosis and therapy. Chen et al. developed hydroxyapatite nanorod probes doped with Eu^{3+} and Gd^{3+} ions, achieving targeted fluorescence, magnetic resonance imaging, and CT imaging of gastric cancer [56]. These nanorods were loaded with ibuprofen hexane, enabling simultaneous imaging and drug release therapy with excellent results. They also developed Eu^{3+}/Gd^{3+}-doped calcium phosphate nanovesicles via copolymer-assisted synthesis, enabling long-term drug release and near-infrared fluorescence and magnetic resonance imaging of tumors.

Ling et al. successfully prepared an intelligent responsive hollow tubular assembly based on bismuth oxycarbonate that can be cleared by the kidneys, and developed it as an efficient and safe nano diagnostic and therapeutic agent for CT imaging mediated radiochemotherapy combined with tumor treatment [57]. They first synthesized ultra small sized bismuth oxycarbonate nanoparticles, and then assembled them into hollow bismuth oxycarbonate nanotubes using solvothermal method. Compared to small-sized nanodots, hollow bismuth oxycarbonate nanotubes have a longer circulating time in the body and are more likely to leak into the blood vessels at the tumor site, subsequently achieving tumor enrichment. Interestingly, this hollow nanotube like assembly will slowly dissociate and assemble into nanoparticles in the slightly acidic environment of tumor tissue, which will then spread to the blood and be cleared by the kidneys, to some extent avoiding the potential toxicity issues caused by long-term accumulation of nanomaterials in the body. Based on this hollow bismuth oxycarbonate nanotube, they continued to develop a nanosystem for synergistic tumor radiotherapy and chemotherapy. On the one hand, under X-ray irradiation, bismuth oxycarbonate nanotubes enriched in the tumor site can achieve tumor specific CT imaging and radiation sensitization. On the other hand, they utilized the characteristic of large specific surface area of hollow nanotubes to load doxorubicin after hydrochloric acid removal inside the pipeline. Both cellular and animal experiments have confirmed that compared to a single radiotherapy or chemotherapy method, the combination of radiotherapy and chemotherapy can significantly increase the levels of reactive oxygen species (ROS) in tumor cells and worsen DNA damage, ultimately inducing tumor cell apoptosis. It can be seen that this intelligent responsive tubular assembly of bismuth oxycarbonate provides a new approach for constructing efficient and non-toxic nano diagnostic and therapeutic formulations.

Liu's group developed a liposome nanoprobe (Lipo@HRP&ABTS) loaded with horseradish peroxidase (HRP) and its substrate ABTS simultaneously [58]. In the environment where hydrogen peroxide exists, this probe has high-intensity near-infrared light absorption, which can not only complete the color reaction commonly found in biochemical assays, but also realize photothermal therapy of tumors. In addition, it can participate in photoacoustic imaging of hydrogen peroxide-related inflammation, and its detection limit can reach submicromolar level.

Nanoparticle-labeled series of stem cell probes

Stem cell technology and nanotechnology are rapidly developing technologies in two different fields. In recent years, these two fields have intersected each

other, and a new field has gradually formed, that is, stem cell nanotechnology. Applying nanotechnology to the research and development of stem cells can solve a series of problems in stem cell research and promote stem cell technology. There are two types of stem cells, embryonic stem cells and adult stem cells. Embryonic stem cells refer to a type of cells that originate from embryos, are in an undifferentiated state, can self-differentiate and self-renew for a long time, and have the potential to differentiate into various tissue cells under certain conditions.

Adult stem cells are undifferentiated cells present in a differentiated tissue that are capable of self-renewal and specialization to form the cells that make up that type of tissue. Adult stem cells exist in various tissues and organs of the body. Adult stem cells in adult individual tissues are mostly in a dormant state under normal conditions, and can exhibit varying degrees of regeneration and renewal capabilities in pathological conditions or induced by external factors. Since the use of human fibroblasts to successfully induce pluripotent stem cells in 2007, human pluripotent stem cells (iPS) cells can be produced in large quantities, providing a sufficient source of cells for the treatment of human diseases and tissue repair.

Ruan et al. isolated bone marrow stromal stem cells from the bone marrow, and identified the bone marrow stromal stem cells, and then used fluorescent magnetic nanoparticles to label the bone marrow stromal stem cells, and injected them into the nude mouse model bearing gastric cancer through the tail vein [59]. The results showed that: after injection, within 2 hours, the bone marrow stromal stem cells labeled with fluorescent magnetic nanoparticles gathered in the tumor site in the body, and 14 days after injection, there was still a strong fluorescent signal in the tumor site in the body. Focusing on tumor sites, their results show for the first time that BMSCs labeled with fluorescent magnetic nanoparticles can actively seek out gastric cancer cells *in vivo*. Utilizing the characteristics of fluorescent magnetic nanoparticles, fluorescence imaging and nuclear magnetic resonance imaging of the tumor site were realized. Using an external magnetic field, local magnetothermal treatment of tumors was also realized, which significantly inhibited the growth of tumors and prolonged the survival time of tumor-bearing mice. They also studied the mechanism of bone marrow stromal stem cells targeting gastric cancer *in vivo*, and the CXCL12-CXCR4 and CCL19-CCR7 axis played an important role.

They also used DiR near-infrared fluorescent dye to label mouse embryonic stem cells. Using a small animal imaging system, they observed that DiR-labeled embryonic stem cells could actively target and recognize gastric cancer cells *in vivo*, and realized near-infrared fluorescence imaging of gastric cancer cells *in vivo* [60]. It was also demonstrated that the CXCR4-CXCL12 axis plays a major role in targeting gastric cancer *in vivo*. They transfected four genes, Oct4, Sox2, LIN28, and Nanog, into 293T cells by using dendrimer-modified magnetic nanoparticles, and successfully prepared human iPS under conditioned medium. The culture medium of iPS cells cultured for 72 hours was also collected and co-cultured with gastric cancer cell MGC803 cells. The results showed that the growth of gastric cancer cells was inhibited. This result indicated that the products secreted by iPS cells had the function of inhibiting the growth of gastric cancer cells. They also used fluorescent magnetic nanoparticles to label human iPS cells, and injected them into tumor-bearing nude mice by tail vein injection [61]. The results showed that human iPS cells gradually gathered around gastric cancer cells *in vivo*. Targeted fluorescence imaging and MRI. Under the action of an external magnetic field, they also used a thermal imager to measure the local temperature of the tumor above 45 degrees, while the temperature of important organs was 38 degrees. Therefore, using an external magnetic field to perform magnetothermal therapy on tumors in the body has a certain degree of safety. Experiments show that: using external magnetic field magnetothermal treatment of tumors for one month, the tumors in the treatment group are significantly smaller than those in the control group. Their results showed for the first time that human iPS cells labeled with fluorescent magnetic nanoparticles can actively target and recognize gastric cancer cells *in vivo*, combined with an external magnetic field, they can achieve magnetothermal therapy of tumors *in vivo*. Using the patient's own bone marrow stromal stem cells, or embryonic stem cells, or iPS cells, after being labeled with nanoparticles, can realize both fluorescence imaging and local tumor treatment, which has the prospect of clinical transformation.

RNA nanoparticles for targeted and simultaneous therapy of gastric cancer

Cui Daxiang and others designed and prepared RNA nanoparticles targeting BRCAA1 gene silencing, as shown in Fig. 6 [62].

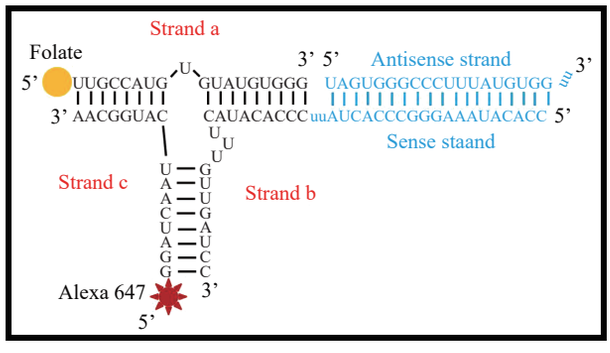

Fig. 6 Structure of RNA nanoparticles. Reproduced with permission from Ref. [62] © 2015 Springer Nature.

Folic acid was selected as the molecule targeting gastric cancer, and the three strands were selected as the siRNA fragment of the BRCAA1 gene; pRNA-3WJ was selected as the carrier to prepare RNA nanoparticles; as shown in Fig. 7, the results showed that: it can actively target gastric cancer, and gradually Accumulate in gastric cancer tissue; significantly inhibit the expression of BRCAA1 gene and protein, inhibit the growth of gastric cancer, and present a significant therapeutic effect. This is the first time that RNA nanoparticles have been shown to significantly inhibit tumor growth in the treatment of living tumors. It also proves that RNA nanoparticles have good biocompatibility, can be efficiently aggregated at tumor sites and have the advantages of targeted therapy.

Integrated nanoprobes for diagnosis and treatment targeting tumor microenvironmental responses

For tumor microenvironment pH, ROS and hypoxic conditions, Yue et al. designed and prepared pH-sensitive self-assembled nanoparticles [63]. The probe first combined D-α -tocopheryl polyethylene glycol 1000 succinate (TPGS) with pH-sensitive cis-

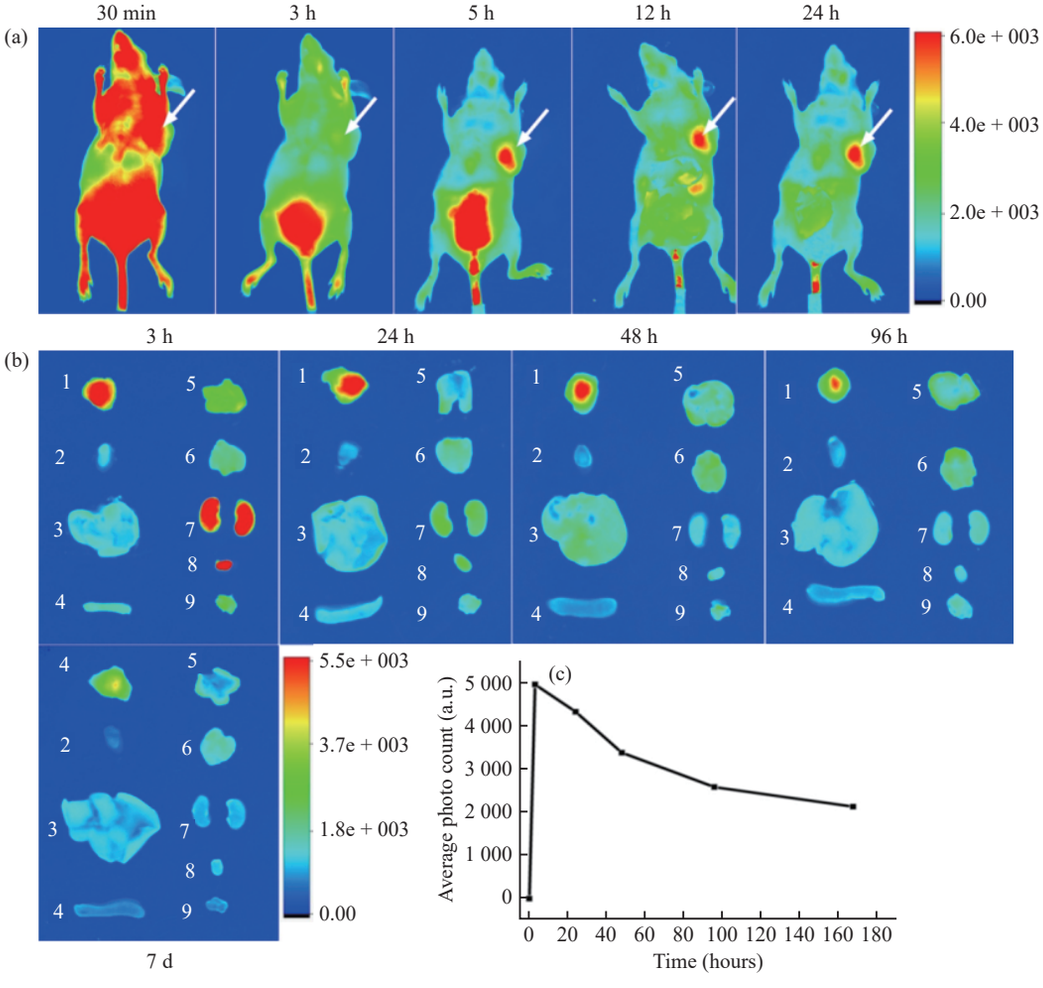

Fig. 7 Fluorescence map of RNA nanoparticles targeting gastric cancer. Reproduced with permission from Ref. [62] © 2015 Springer Nature.

aconic Anhydride-modified doxorubicin (TCAD) is coupled with anti-tumor drugs to form self-assembled micelles, and then Ce6 is loaded into the micelles. After reaching the tumor site, the pH-sensitive site will be broken to release the anti-tumor drugs. At the same time, the near-infrared fluorescence imaging performance of the photosensitizer Ce6 is used to illuminate the tumor under near-infrared light, and simultaneously realize the photodynamic therapy of the tumor. This dual therapy with targeted chemotherapy and light-controlled targeted photodynamic therapy can greatly improve the treatment efficiency of tumors and reduce the side effects of chemotherapy drugs while performing near-infrared fluorescence imaging on tumors. It is expected to greatly increase the cure rate of tumors and improve the quality of life of patients.

The ROS-activated ce6-CPT-UCNP nanoprobe excited by near-infrared light was developed [64]. This nanoparticle is a ROS-sensitive thiol linker, and the chemotherapy drug camptothecin is linked to the thiol linker (abbreviated as TL- CPT). At the same time, the upconversion luminescent nanoparticles (Ce6-CPT-UCNPs) were modified with photosensitizer Ce6, TL-CPT and carboxylated PEG. When irradiated with 980 nm laser, UCNPs emit fluorescence at 645–675 nm. Ce6 can absorb 645–675 nm light to generate ROS. ROS can not only be used in photodynamic therapy, but also cut off the ROS-sensitive thiol linker and release the chemotherapy drug CPT. At the same time, the nanoparticles can be used for fluorescence imaging. *In vivo* imaging experiments show that the nanoparticles can be used for lung cancer in situ imaging; *in vivo* therapy experiments show that Ce6-CPT-UCNPs can be used for combined treatment of photodynamic therapy and chemotherapy under the irradiation of 980 nm laser.

The GNS@$CaCO_3$/ICG probe was developed to achieve pH-responsive drug release, using NIR-induced photodynamic therapy of gold nanostars and photothermal therapy of ICG [65]. Developed tumor cells with high MMP2 expression in the tumor microenvironment, self-assembled nanomedicines of cy5-modified MMP2 peptides, camptothecin, and retinoic acid, and realized targeted delivery and chemotherapy of tumors *in vivo*. The mitochondria-targeted NIR-induced thermosensitive liposomes were developed to realize the combination therapy of photothermal, photodynamic and chemotherapy.

Song et al. synthesized a carboxyl-functionalized Pluronic P123 block copolymer, linked the hypoxia-inducible factor HIF 1-alpha antibody to the prepared block copolymer, and used hydrophobic groups to self-assemble with paclitaxel to form HIF1- The alpha antibody-linked paclitaxel nano-targeted micelles, through cell and animal models, proved that the micelles can achieve efficient targeted delivery and treatment of paclitaxel [66].

With the popularization of the concept of individualized treatment, modern nanomedicine pays more attention to tumor heterogeneity and individualized treatment plan suitable for patients. Nano-diagnosis and treatment preparations have their unique advantages in this respect and have good application prospects. But more importantly, researchers should break the traditional concept and find more efficient treatment methods. For example, physically integrated diagnostic and therapeutic nanoparticles have certain advantages for some applications. In addition to deliver traditional chemotherapy drugs, nanocarriers can also be used to deliver immune agents. Immunotherapy can induce long-lasting and systemic antitumor immune responses, especially for metastatic tumors. Many immunotherapeutic preparations can be effectively delivered to target tissues with improved efficacy and reduced side effects. Although nano-therapeutic preparations have great potential, there are still many difficulties in the process of clinical translation. People should have a deep understanding of the interaction between nanoparticles and tumors, the cooperation between diagnosis and treatment, and pay more attention to the industrial production of nano-preparations, long-term toxicity, and regulatory solutions. In this way, effective individualized treatment can be achieved.

Progress in research on anti-tumor nanomedicines

With the continuous development of nanoscience and tumor biology, the research of antitumor nanomedicines has also made great progress, and 43 nanomedicines have been used for the clinical treatment of tumors so far with good results, such as paclitaxel liposome, adriamycin liposome, duck bile oil emulsion and irinotecan liposome (Table 1). Global biopharmaceuticals are entering the era of nanotechnology. Nanomedicines have certain advantages in tumor therapy, which can improve the

Table 1 Globally marketed nanomedicines and indications

Drug	Indication	Listed countries/ regions	Time to market
AmBisome	Systemic fungal infections	USA	1997
Daunoxome	Boehl sarcoma	USA	1996
DepoCyt	Meninges of malignant	USA	1999
DepoDur	Chronic pain	USA	2004
Doxil	AIDS,multiple myeloma, and ovarian cancer	USA	1995
Inflexal	Flu	Switzerland	1997
Marqibo	Acute lymphoblastic leukemia	USA	2012
Mepact	Osteosarcoma	Europe	2009
Myocet	Metastatic breast cancer	Europe	2000
Visudyne	Macular degeneration	USA	2000
Abelcet	Systemic fungal infections	USA	1995
Amphotec	Systemic fungal infections	USA	1996
Adagen	Adenosine deaminase deficiency	USA	1990
Cimzia	Crohn's disease and rheumatoid arthritis	USA	2008
Neulasta	Febrile neutropenia and hematological malignancies	USA	2002
Oncaspar	Acute lymphoblastic leukaemia	USA	1994
Pegasy	Hepatitis B and C	USA	2002
Peglntron	Hepatitis C	USA	2001
Somavert	Megalakria	USA	2003
Macugen	Macular degeneration	USA	2004
Mircera	Chronic renal failure	USA	2007
Emend	Antemetic	USA	2003
Megace ES	Anorexia and cachexia	USA	2005
Rapamune	Immunosuppressants	USA	2002
Tricor	Hypercholesterolemia and hypertriglyceridemia	USA	2004
Triglide	Boehl sarcoma	USA	2004
Copaxone	Multiple sclerosis	USA	2014
Eligard	Prostate cancer	USA	2002
Genexol	Metastatic breast cancer and pancreatic cancer	USA	2001
Opaxio	Acute lymphoblastic leukemia	USA	2012
Renagle	Osteosarcoma	USA	2000
Zinostatinstimalamer	Metastatic breast cancer and pancreatic cancer	Japan	1994
Kadcyla	Metastatic breast cancer	USA	2013
Ontak	T-Cell Lymphoma	USA	2005
Fendex	Liver and splenic lesions MRI	USA	1996/2008
Feraheme	Iron deficiency anemia (IDA) in chronic kidney disease	USA	2009
NanoTherm	Glioblastoma, prostate, and pancreatic cancer.	Europe	2013
Gendicine	Tumour	China	2003
Rexin-G	Solid tumour	The Philippines	2007
Diprivan	Calm hypnosis and anesthesia	USA	1989
Estrasorb	Hormone replacement therapy for menopause	USA	2003
Fungizone	Systemic fungal infections	USA	1966
Abraxane	Metastatic breast cancer and	USA	2005

tumor targeting of drugs, enhance the stability of drugs, improve the kinetic behavior of drugs, improve bioavailability, and reduce the toxic side effects of drugs, etc. By constructing nanomedicine delivery systems with pH responsiveness, enzyme responsiveness, reduction responsiveness, and responsiveness to external stimuli such as light, heat, and magnetism, it can also achieve precise control of drug release. In addition, nanomedicines can also encapsulate a variety of imaging, diagnostic and therapeutic drugs to achieve drug co-delivery and co-localization and realize multi-functional multi-therapeutic combination applications. At present, the research on antitumor nanomedicines is mainly focused on targeted drug delivery, regulation of tumor microenvironment, tumor gene therapy, and tumor immunotherapy.

Tumor-targeted delivery of nanomedicines

The discovery of chemotherapeutic drugs has made great contributions to the clinical treatment of tumors, but the severe toxic side effects of chemotherapeutic drugs pose a challenge to the treatment of tumors. Nanotechnology-based drug delivery systems have unique advantages in the targeted delivery of antitumor drugs. Tumor tissues have enhanced permeability and retention effect (EPR effect), and nanomedicines can be passively targeted to tumor sites through the EPR effect. Active targeting is to use the affinity of targeting molecules for specific cells, tissues, etc. to achieve targeted delivery of nanomedicines to tumors, and the commonly used targeting moieties include antibodies and their fragments, targeting peptides, membrane penetrating peptides, aptamers, integrins, and their ligands.

With the in-depth research on new nanocarrier targeting systems by domestic and foreign scholars, a series of new active targeting nanomedicine delivery systems have been developed, among which, bionanomedicines represented by lipoproteins, albumins, and cell membranes have become a hot spot for research due to their high targeting and biocompatibility. Huang et al. constructed a lipoprotein mimetic nanomedicine-encapsulated transcriptional activator 5 siRNA to penetrate the blood-brain barrier, target glioblastoma, downregulate transcriptional activator 5 expressions, and promote apoptosis in glioblastoma [67]. Zhang et al. modified liposomes by using a short non-toxic peptide, Aβ25-35, which specifically interacts with the lipid-binding structural domain of apolipoproteins to expose the receptor-binding structural domain of apolipoproteins for brain-targeted delivery. The binding of albumin to the receptor gp60 on the surface of vascular endothelial cells mediates transmembrane drug transport and increases drug accumulation in tumor cells [68]. Yang et al. attached the photosensitizers dihydro porphyrin e6 (Ce6) and oxaliplatin to human serum albumin and coupled them to a 100–150 nm nano-drug delivery system (HCHOA) via a lack of oxygen-sensitive azobenzene, respectively. When the HCHOA is targeted to the tumor site, it rapidly breaks down into nanoparticles less than 10 nm in diameter under the action of the oxygen-depleted tumor microenvironment, significantly enhancing deep tumor penetration and achieving the synergistic anti-tumor efficacy of photodynamic therapy and chemotherapeutic agents [69].

In the last five years, several advances have been made in cell membrane-based bionanosystems for the targeted delivery of drugs to tumor sites. Commonly used cell membrane carriers have been studied mainly in erythrocyte membranes, mesenchymal stem cells, monocytes/macrophages, and tumor cell membranes. Erythrocyte membrane-modified nanosystems with long circulation can accumulate at tumor sites through the EPR effect, and the modification of tumor-targeting groups on the surface of erythrocyte membranes can further improve drug delivery efficiency (Fig. 8). Chai et al. prepared the core of PLGA-encapsulated photosensitizer ICG and extracted the cell membrane of MCF-7 cells to successfully prepare tumor cell membrane-encapsulated bionanomedicine delivery systems (ICNPs). ICNPs have homologous targeting of tumor cells, photothermal responsiveness, and fluorescence/photoacoustic imaging properties, enabling high spatial resolution and depth penetration and real-time monitoring of dynamic distribution *in vivo* while combining with photothermal therapy to improve the therapeutic effect on tumors [70]. Zhang et al. developed a macrophage membrane-encapsulated tumor microenvironment responsive release targeted nano-delivery system, in which macrophage membranes can be shed by extracellular microenvironment stimulation and the kernel is taken up by tumor cells and the drug is rapidly released under intracellular pH conditions, improving the anti-tumor therapeutic effect [71]. Fang et al. reviewed the

advance of cell membrane-coated nanoparticles (CNPs) for biomedical applications such as drug delivery, phototherapy and immunotherapy. CNPs own obvious advantages such as improved biocompatibility, immune evasion and tumour targeting. CNPs may precisely deliver loaded drug for tumor therapy, owns clinical translational values [72].

Nanodrugs regulate tumor microenvironment

Tumor cells, macrophages, dendritic cells, fibroblasts, T cells, B cells, myeloid inhibitory cells, extracellular matrix, cytokines, proteases, blood vessels and lymphatic vessels together constitute a complex tumor microenvironment which has the characteristics different from normal tissues，such as tissue hypoxia, slightly acidic environment, abnormal angiogenesis and high reducibility. The tumors can evade recognition and attack by the immune system through various mechanisms to achieve tumor growth and metastasis. Therefore, the regulation of tumor microenvironment has become a research hotspot in tumor therapy and the current application of nanotechnology targeting tumor microenvironment mainly focuses on regulating tumor hypoxia microenvironment and tumor-associated macrophage.

The hypoxic microenvironment inhibits the anti-tumor activity of related immune cells and promotes the proliferation, invasion, and metastasis of tumor cells. The use of nanotechnology to achieve targeted regulation of tumor hypoxic microenvironment to increase oxygen content at tumor sites has become an emerging strategy for cancer treatment. Liu et al. synthesized transmucosal delivery nanoparticles co-assembled by fluorinated chitosan with sonosensitizer meso-tetra(4-carboxyphenyl)porphine (TCPP)-conjugated catalase (Fig. 9) to alleviate hypoxia in tumor tissue microenvironment by catalyzing tumor endogenous H_2O_2 through catalase [73].

Chen et al. developed a fluorocarbon (FC)-chain-mediated hollow mesoporous organosilica nanoparticles to simultaneously transport sonosensitizer and oxygen, in the PANC-1 pancreatic cancer model the nanoparticles can effectively deliver oxygen to the hypoxic part of the tumor [74].

Tumor-associated macrophages (TAMs) promote not only the generation and metastasis of tumors, but also the angiogenesis and implantation of metastatic tumor cells in remote tissues. Therefore, TAMs are key targets for inhibiting tumor proliferation and metastasis [75]. Zhao et al. aimed at the high expression of cysteine rich acidic secretory protein (SPARC protein) and Mannose receptor in colon cancer cells and M2 type TAMs, prepared mannosylated albumin nanoparticles to achieve dual targeting of colon cancer cells and TAMs. These nanoparticles can upregulate the levels of ROS in tumor cells, enhance tumor cell apoptosis, reverse M2 type TAMs, and at the same time inhibit the generation of tumor neovascularization and the proliferation of drug-resistant colon cancer cells [76]. Qian et al. constructed a double targeted nanoparticles based on α-peptide (a scavenger receptor B type 1 (SR-B1) targeting peptide) linked with M2pep (an M2 macrophage binding peptide). The nanoparticles can specifically block the survival signal of TAMs and significantly eliminate M2 like

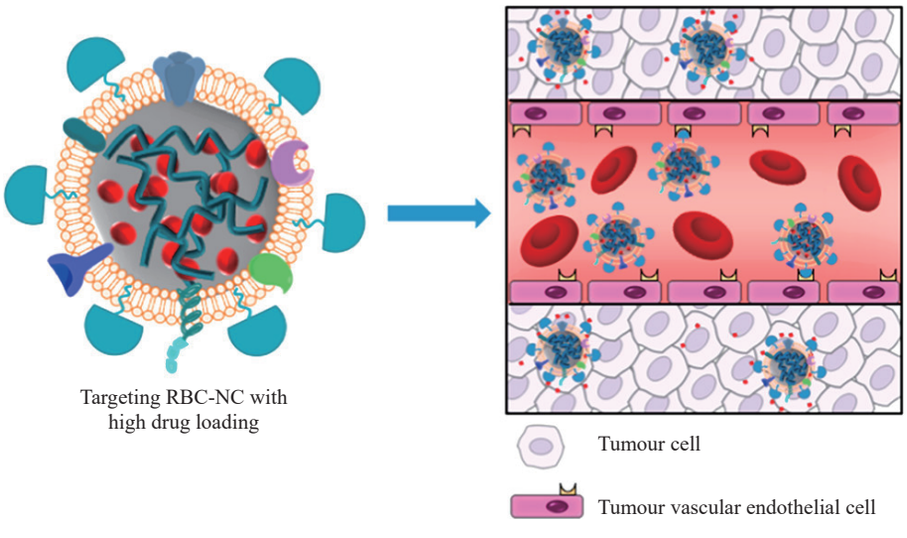

Fig. 8 c(RGDyK)-targeted peptide-modified erythrocyte membrane-encapsulated nanocrystal drug delivery system. Reproduced with permission from Ref. [70] © 2019 American Chemical Society.

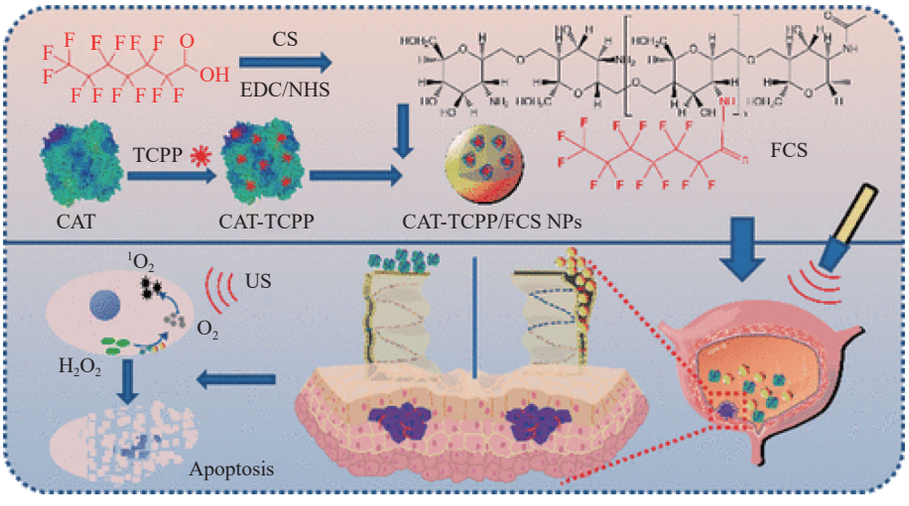

Fig. 9 Fluorinated chitosan nanoparticles based on Catalase alleviate tumor hypoxia microenvironment and improve tumor ultrasound treatment effect. Reproduced with permission from Ref. [73] © 2020 American Chemical Society.

TAMs to reduce the growth of melanoma by loading anti-colony stimulating factor-1 receptor (anti-CSF-1R) small interfering RNA (siRNA) on the M2NPs [77].

Shi et al. constructed a new nano reprogramming drug loading system that can reverse the M2 type TAMs to M1 type macrophages (Fig. 10) while inhibiting the proton pump and proteolytic activity of lysosome, and enhance the antigen presentation and T cell function of TAMs by promoting the release of tumor related antigens in the cytoplasm to clear tumors, inhibit metastasis and prevent recurrence [78].

Tumor gene therapy based on nanodrugs

Tumor gene therapy provides a new method for tumor treatment by delivering relevant gene fragments into tumor cells. Gendicine is the first gene therapy drug approved for marketing in the world which based on the recombination between adenovirus and tumor suppressor gene (human p53 gene) [79]. The emergence of CRISPR/Cas9 gene editing tools has brought new opportunities for the treatment of tumor gene drugs. Since the first clinical trial of CRISPR/Cas9 for tumor treatment was conducted by Sichuan West China Hospital in 2016 [80], multiple tumor gene therapies based on CRISPR/Cas9 technology have entered clinical trials. The commonly used gene drug carriers are viral and non viral vectors, viral vectors are mainly adenoviral vectors with high transfection efficiency, but there are potential risks of immunogenicity, cytotoxicity and genetic recombination, non viral vectors avoid potential risks of viral vectors while delivering gene drugs.

Gene therapy based on nanotechnology has

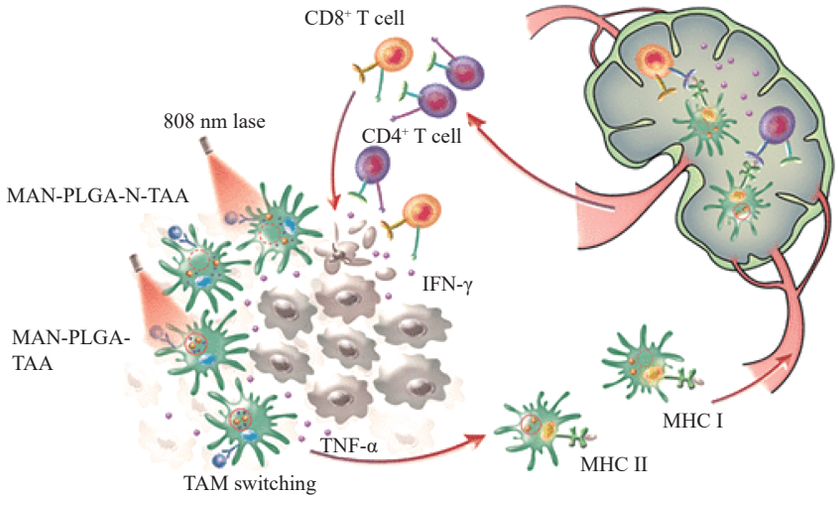

Fig. 10 Reprogramming nanodrug reverses M2 type TAMs. Reproduced with permission from Ref. [78] © 2018 American Chemical Society.

significant advantages in tumor treatment. Nanodrugs can protect gene drugs from being degraded by proteases before reaching the target cells while avoiding the potential risks of viral vectors. With the development of nanotechnology more new and efficient nano gene carriers are being applied in the research of tumor treatment. Kim et al. prepared lipid nanoparticles containing C-24 alkyl phytosterols as carriers for mRNA transport. The polyhedral shape of the nanoparticles exhibited better cellular uptake and retention capabilities [81].

Pi et al. used nanotechnology to change the direction of arrow shaped RNA to control the nucleic acid aptamer or folic acid anchored on the extracellular vesicles to achieve targeted delivery to cells, which showed excellent gene delivery and tumor growth inhibition ability in prostate cancer xenograft models, situ breast cancer models, and patient derived colorectal cancer xenograft models [82].

Pan et al. designed a near-infrared light responsive nanocarrier for CRISPR-Cas9 based on upconversion nanoparticles (Fig. 11). The upconversion nanoparticles, acting as "nano energy converters", convert near-infrared light into local ultraviolet light and cleave the photosensitizers to accurately release Cas9-sgRNA. Through exogenous control methods, this study provided enormous potential for targeted gene editing and tumor therapy in deep tissues [83].

Nanodrug-mediated tumor immunotherapy

Immunotherapy for tumors has become one of the important strategies for tumor treatment and has good effect in preventing tumor growth, recurrence and metastasis. Currently, monoclonal antibody drugs including immune checkpoint inhibitors such as PD-1/PD-L1 monoclonal antibody, CTLA-4 monoclonal antibody, chimeric antigen receptor T cells represented by Kymriah and autologous cell tumor vaccines represented by Sipuleucel-T have been clinically applied and have achieved certain effects [84]. Tumor immunotherapy has brought a new revolution in the field of tumor treatment, but due to the disadvantages of tumor immunotherapy such as low accumulation at tumor sites and low response rate of patients in general, and also due to the tumor microenvironment in which tumor cells are located, they can evade the recognition of immune system through various mechanisms, which leads to the failure of tumor immunotherapy. Therefore, conventional tumor immunotherapies cannot fully meet clinical needs yet, and the search for novel tumor immunotherapies with high efficiency and low toxicity is still a major problem that needs to be addressed urgently.

With the rapid development of nanomedicines, nanodrug delivery systems have been widely used in the study of tumor immunotherapy and have made some progress. Hou et al. used acid-activatable cationic micelles to co-deliver photosensitizers (PS)

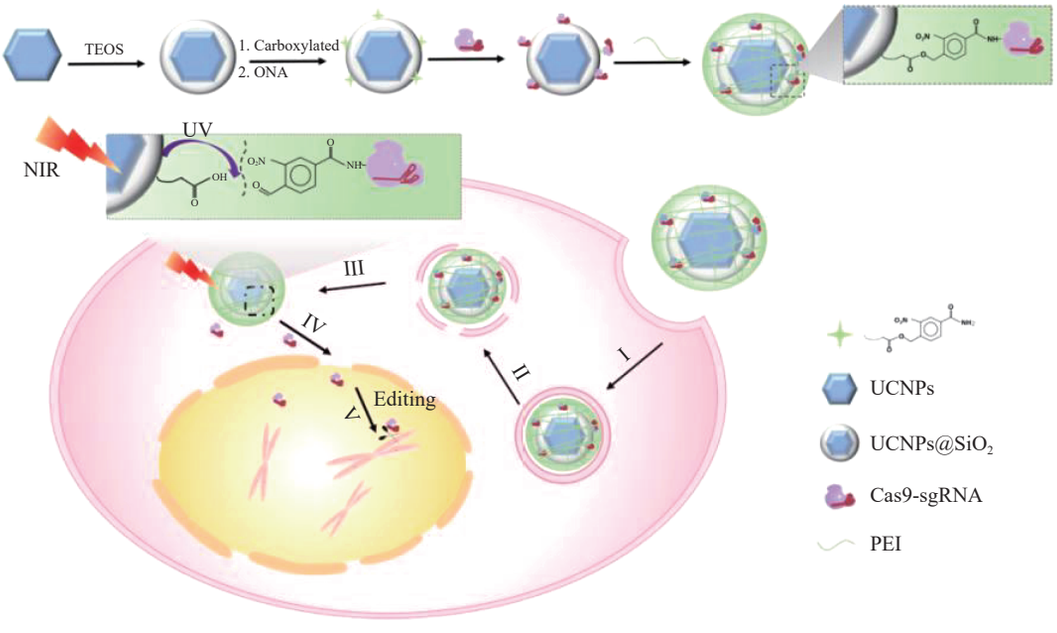

Fig. 11 Response Release of Cas9-sgRNA Upconversion Nanocarriers Controlled by Near Infrared Light. Reproduced with permission from Ref. [83] © 2019 John Wiley & Sons.

and small molecule interfering RNA (siRNA) to enhance photodynamic therapy-induced cancer immunotherapy by inhibiting PD-L1 expression in tumor cells in a B16- F10 melanoma xenograft tumor model showed significantly enhanced efficacy in inhibiting tumor growth and distant metastasis [85]. Mannose-modified tumor vaccines were constructed by Yang et al [86].

The polymeric material PLGA encapsulated with the Toll-like receptor-7 agonist imiquimod and coated with tumor cell membranes as antigen can promote the maturation of antigen-presenting cells and enhance the tumor immune effect [87]. Zhou et al. constructed a nanosystem encapsulating oxaliplatin prodrug and PEGylated photosensitizer (Fig. 12), which can specifically accumulate into the tumor and penetrate deep into the tumor, rapidly release the drug in response to the tumor microacidic environment and enzymes, induce immunogenic cell death in tumor cells, further promote dendritic cell maturation in combination with CD47 blocker, promote antigen presentation by DC cells, and effectively inhibit the growth of primary and secondary tumors, inhibits tumor metastasis and prevents tumor relapse [88].

The response rate of single tumor immunotherapy is low and the immune activation capacity is still inadequate, which still has its limitations in clinical research and application, therefore, tumor immunotherapy based on photodynamic, photothermal, radiotherapy and other means has become the focus of research. Rixe et al. prepared a hybridized protein oxygen nanocarrier loaded with photosensitizer Ce6 by hybridizing human serum albumin with hemoglobin through intermolecular disulfide bonds. The nanoparticles enhanced $CD8^+$ T-cell infiltration in tumors, increased PDT-induced

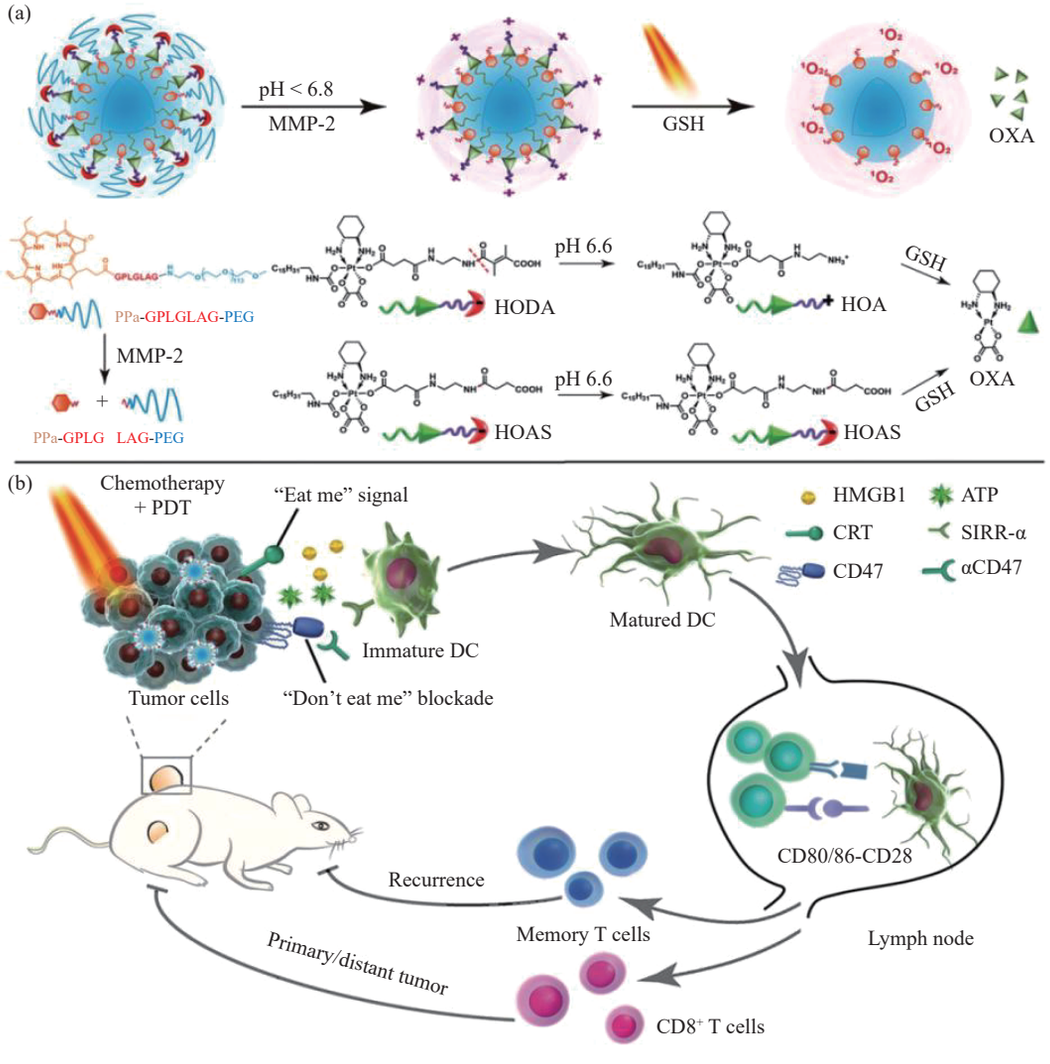

Fig. 12 (a) Acid-sensitive and MMP-2 enzyme dual-sensitive nanoprecursors; (b) nanoprecursors combined with anti-CD40 inhibit tumor proliferation, recurrence and distal metastasis. Reproduced with permission from Ref. [88] © 2019 WILEY-VCH Verlag.

immunogenic cell death, activated dendritic cells, T-lymphocytes and natural killer cells *in vivo*, inhibited primary tumor proliferation and effectively suppressed distant tumors and lung metastases [89]. Li et al. developed a multifunctional nanoplatform for enhancing tumor immunotherapy in combination with phototherapy and chemotherapy, in which chemotherapeutic agents promote CTL infiltration and M1 macrophage polarization, and subcutaneous injection of PD-L1 antibody further enhances anti-tumor efficacy [90]. Wang et al. constructed an antibody drug delivery system based on PD-L1 immune checkpoint antibody (αPD-L1) to co-deliver photosensitizer molecules ICG and αPD-L1 (Fig. 13), which increased the intratumoral accumulation of antibody nanoparticles; prolonged the retention time to achieve slow release of αPD-L1 antibody in tumors; induced inflammatory response at tumor sites in synergy with the photodynamic effect of ICG; and enhanced the immunogenicity of tumors [91].

Radiation therapy enhances immune recognition of "cold" tumors, leading to a diverse anti-tumor T cell response. Cheng et al. reviewed the advances of IL-12 used as an in situ cancer vaccine component for tumor therapy. Interleukin-12 (IL-12) can stimulate T and natural killer cell activity and induces interferon gamma production. IL-12 has advantages as in situ vaccine for tumor immunotherapy combined with other cancer treatment modalities such as radiation therapy, owns application prospect in clinical cancer therapy [92].

American scientists have developed BXQ-350 (composed of nanobubbles formed by the body's naturally expressed protein, activator protein C, and the fat molecule DOPS), a therapeutic drug that selectively targets and kills cancer cells without affecting surrounding healthy tissue, and has entered Phase I clinical trials. It is also able to cross the blood-brain barrier, which makes this nano-drug highly effective in treating malignant brain tumors [93].

Nanodrug-mediated tumor radiation therapy

Radiation therapy is a local treatment method using radiation to treat tumors, which has become one of the most common and effective treatments for malignant tumors in clinical practice. However, radiation therapy still has disadvantages such as high radiation dose, high side effects on healthy tissues, especially strong radioresistance of tumor cells. With the development of nanomedicine, multifunctional nanomaterials with good biocompatibility and safety have received wide attention and shown good potential in tumor radiation therapy applications. The introduction of them as radiotherapy sensitizers or radiotherapy sensitizer carriers into tumor radiation therapy has the potential to overcome many difficulties currently limiting tumor radiation therapy and provides new opportunities to promote the further development of radiation therapy.

The ideal radiotherapy sensitizer should have the following requirements: First, radiotherapy sensitizers should have good biocompatibility and safety to ensure that the sensitizer can enter the tumor

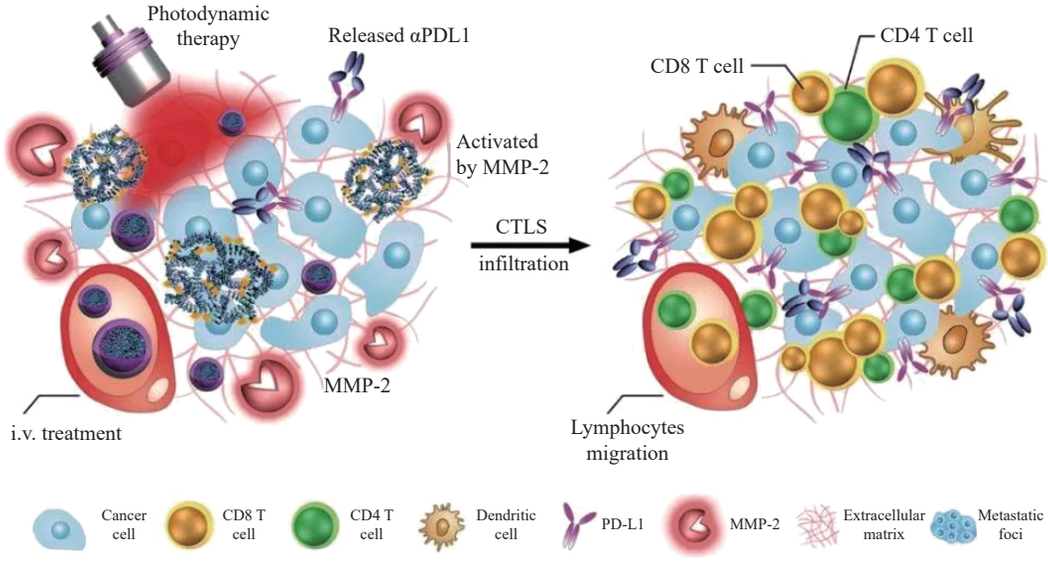

Fig. 13 PD-L1 immune checkpoint antibody synergizes with photodynamic therapy to enhance tumor immunogenicity. Reproduced with permission from Ref. [91] © 2021 John Wiley & Sons.

tissue and has little non-toxic or toxic effect on healthy tissue and normal cells; next, radiotherapy sensitizer should have a suitable biological half-row duration to ensure the effective concentration of the sensitizer and the drug in the body; moreover, because the microenvironment of the tumor area is different from the normal tissue, the sensitizing agents should be specific, targeted to sensitized tumor cells, which have little or no sensitizing effect on normal cells; in addition, it should easily cross the blood-brain barrier in brain tumors.

Commonly used sensitized nanomaterials are precious metal nanomaterials such as gold, silver and platinum; nanomaterials containing heavy metal elements such as gadolinium, hafnium, tantalum, tungsten, bismuth; ferrite nanomaterials; semiconductor nanomaterials, etc. In 2017, by integrating the radiosensitization characteristics of gold nanorods and the anti-tumor activity of selenium nanoparticles, Yu's research team designed the gold/selenium nanocomposite system, and used the surface modified dual-targeting molecules as a new nano-radiotherapy sensitizer, to realize the tumor-targeted chemoradiotherapy method [94]. This nanocomposite system showed excellent tumor targeting capability, good biosafety, efficient radiotherapy sensitization, and significant antitumor effects. AGuIX, developed by The French company HTherAguix, has entered the clinical experiment of brain metastases [95]. However, the research on nanosensitizer is still in its infancy, both promising and has many problems to be solved.

In short, chemotherapy, radiotherapy and other means have been widely used in the clinical treatment of tumors, but conventional chemotherapy and radiotherapy and other means have serious toxic and side effects, and are easy to lead to the recurrence and metastasis of tumors, leading to the failure of clinical treatment. Emerging tumor immunotherapies such as monoclonal antibodies, immune checkpoint inhibitors, and tumor vaccines are also facing great challenges such as low drug delivery efficiency and poor patient responsiveness. Nanodrugs can target the delivery of drugs to tumor sites, improve the efficacy, and become the research hotspot of anti-tumor drugs. With the continuous research of tumor biology, people have a deeper understanding of the mechanisms of tumor initiation, development and metastasis. Based on the development of tumor biology, genomics and nano-science, the research and development of new nano-drugs have greatly enriched the means of tumor treatment, with advantages in targeted delivery of drugs, regulation of tumor microenvironment, delivery of gene drugs, enhancing tumor immunotherapy and other aspects, providing a new opportunity for tumor treatment.

Summary and Outlook

When nanotechnology first emerged in the 1980s and 1990s, it was more focused on its application in electronic products. It was not until around 2000 that the important role of nanotechnology in medical research and clinical applications was realized. Afterward, in order to seize the commanding heights of nanotechnology and nanomedicine, governments around the world invested heavily in their research and development. For example, in 2005, the United States announced the launch of the "Cancer Nanotechnology" program and established the "Cancer Nanotechnology Federation". From 2005 to 2006, the National Institutes of Health (NIH) funded the establishment of 20 Nanomedicine research centers in the USA and carried out the NIH Nanomedicine Roadmap Initiative, the National Cancer Institute Nanotechnology Alliance, and the National Heart, Lung, and Blood Institute Nanotechnology Excellence Program (PEN), The Institute of Environmental Health Sciences (NIEHS) Nanohealth Enterprise Initiative and other major plans of Nanomedicine truly carry out large-scale systematic research. In addition, research on nanotechnology and nanomedicine has also become a new hot topic in developed countries such as Japan, Germany, and the UK [96]. According to the report "Nanobiotechnology: Applications and Global Markets" published by BCC Research in January 2011, the market for nanobiotechnology products reached $19.3 billion in 2010, with medical applications (including drug delivery and microbicides) occupying the current market, with sales reaching $19.1 billion.

The United States has identified nanomedicine as a breakthrough focus, mainly focusing on its applications in molecular biology, early disease detection and treatment, nano-drug delivery, nano biomimetics, key nanotechnology in tissue engineering, and nanobiological devices. The NIH of

the USA launched the "Cancer Nanotechnology Program" in 2005, aiming to integrate nanotechnology, cancer research, and molecular biomedicine, and achieve its goal of eliminating cancer deaths and pain by 2015 through a series of research programs [97, 98]. The key research content is to utilize nanotechnology, mainly including nanoparticle material technology and nanosensor technology, to form some new early diagnosis, warning and treatment technologies and nanodevices for malignant tumors. Although the "Cancer Nanotechnology Plan" of the United States is far from reaching the original goal, this technology has cultivated a large number of talents engaged in nanotechnology research and development, promoted the development of Nanomedicine technology, and laid the foundation for the USA to occupy a dominant position in the field of nanotechnology precision medicine [96, 99].

Japan is one of the earliest countries in the world to formulate nanotechnology plans and the largest investor in the development of microelectromechanical systems using nanotechnology. The "Nanotechnology Comprehensive Support Plan" implemented by Japan aims to maximize the potential capabilities of various research institutions, organize joint public relations, build special research facilities, and promote the research and development of nanotechnology [100]. The research focus of its nano biological devices includes micro nanosystems for diagnosing and treating lesions in the body and nano biomimetic materials. The Japanese government has implemented the "Nanomedical Device Development Plan" since 2002, aiming to develop various micro medical devices such as millimeter-scale endoscopes, ultra-fine cell imaging devices that can observe protein activity and drug delivery systems that can efficiently deliver drugs to lesions, aiming to achieve practicality within 5–10 years. To this end, the Japanese government has set up a "cutting-edge science and health care integration research institution" at Waseda University. The government invests 800 million yen every year to carry out research and development of nano diagnostic technology and devices. It is an international leader in Artificial hearts, Microfluidics systems, robots, nano drug stents, molecular imaging equipment, etc.

The EU launched the 7th Framework Plan in 2006, committed to enhancing the international status of nanotechnology. The research focuses proposed by Germany include drug delivery systems, biomolecular manipulation, and detection technologies, nanoelectronics with biological entities, and research on biological entity interfaces and detection technologies. At present, the research focuses on the nano-targeted drug delivery system that can destroy tumor cells, the nano biosensor used to diagnose the formation of antibodies in human blood after infection, and the nanodevices used to treat cancer and cardiovascular diseases.

Therefore, it is an international trend to strengthen the research and development of nano biological devices based on nanomaterials and nano effects and actively promote their industrialization, which will strongly promote the development of nano Precision medicine, and have important practical significance to enhance the international status in the field of nanobiotechnology, with huge economic and social benefits [101, 102].

Nanobiotechnology refers to the nanotechnology that studies life phenomena. It is an emerging discipline formed by the cross-penetration of nanotechnology and biology and involves a comprehensive interdisciplinary field of physics, chemistry, quantum science, medicine, pharmacy, materials science, electronics, computer science, and many other fields [103, 104]. It has broad application and industrialization prospects in the field of biomedicine and will be applied in disease diagnosis, preventive treatment, tissue and organ repair, replacement, and other aspects that play an important role, which is the core research and development content of nano Precision medicine [105–107].

Nanobiotechnology has developed rapidly and achieved a series of gratifying results in a short period of time. Research results have emerged endlessly, with some achievements entering or approaching the stage of industrialization. For example, since the implementation of NNI in the United States, breakthroughs have been made in the basic research of nanotechnology. In terms of applied research and product development, the four hot areas of semiconductor chips, cancer diagnosis, optical new materials, and biomolecular tracking have also developed rapidly [108]. In terms of disease detection, it has been proven that magnetic nanoparticles, gold nanoparticles, and other

nanoparticles have higher sensitivity in detecting disease markers, which is greatly helpful for the early diagnosis of many diseases such as tumors; In the treatment of diseases, it has been found that drugs using nanoparticles as carriers can reduce side effects and increase lesion targeting [109]. At present, doxorubicin liposome nanomedicine and paclitaxel albumin nanomedicine have entered the market, and dozens of nanomedicines are currently undergoing clinical trials, which are expected to enter the market for sale soon. From the current progress, it is expected that in the next 5–10 years, more nano-detection technologies and nano drugs will be used in clinical practice, saving patient lives [110]. For example, in March 2010, researchers from the California Institute of Technology and other institutions in the United States successfully developed a kind of micro nanoparticles, which can enter the tumor through the blood flow of patients, and then release drugs, which can accurately block the cancer gene named RRM2. It proved that the treatment method of RNAi technology can work in humans, providing preliminary evidence. Scientists from the University of California, San Diego, Santa Barbara, and Massachusetts Institute of Technology have developed a novel "cocktail therapy" at the nanoscale, which can simultaneously locate tumor cells in the blood and release anticancer drugs to achieve the goal of eliminating tumors. Daxing Cui's research team collaborated with Peixuan Guo from the USA to design and prepare RNA nanoparticles targeting the BRCAA1 gene. The experiment showed that the prepared RNA nanoparticles have good biocompatibility, can target gastric cancer cells *in vivo*, inhibit the growth of gastric cancer, and have clinical application prospects.

Stem cell research and development has been a global frontier hotspot. The combination of nanotechnology and stem cell technology has resulted in the emergence of a new research field, namely stem cell nanotechnology [111, 112], which is also a significant research direction in the field of nano-precision medicine: applying nanotechnology to the research and development process of stem cells can solve several problems in stem cell research and promote the rapid development of stem cell technology. Stem cells are divided into two types, namely embryonic stem cells and adult stem cells [113, 114]. Embryonic stem cells are a type of cells that originate from embryos, are undifferentiated and capable of long-term self-differentiation and self-renewal, and have the potential to differentiate and form various tissue cells under certain conditions. By labeling mouse embryonic stem cells with the near-infrared fluorescent dye DiR and using the small animal imaging system afterward, it can be observed that DiR-labeled embryonic stem cells can actively target and recognize gastric cancer cells *in vivo*, realizing near-infrared fluorescence imaging of gastric cancer cells *in vivo*. And it also demonstrates that the CXCR4-CXCL12 axis plays a major role in the process of embryonic stem cells targeting gastric cancer *in vivo* [115, 116]. The effect of single-walled carbon nanotubes on the proliferation and differentiation of embryonic stem cells was also investigated. The dendrimer-modified carbon nanotubes could enter embryonic stem cells and stimulate the growth and development of embryonic stem cells [117]. Cui et al. transfected four genes (Oct4, Sox2, LIN28, and Nanog) into 293T cells by using dendrimer-modified magnetic nanoparticles, and successfully prepared human induced pluripotent stem (iPS) cells in a conditioned medium, and also collected the medium of iPS cells cultured for 72 hours, and co-cultured with gastric cancer MGC803 cells, liver cancer cell lines, and breast cancer cell lines, etc. The results showed that the growth of gastric cancer cells and others was inhibited, indicating that the product secreted by iPS cells has the function of inhibiting the growth of gastric cancer and other cells61. They also used fluorescent magnetic nanoparticles to label human iPS cells and injected them into tumor-bearing nude mice through the tail vein. The results demonstrated that human iPS cells gradually gathered around gastric cancer cells *in vivo*, and that targeted fluorescence imaging and nuclear magnetic resonance imaging of gastric cancer cells were realized by utilizing the properties of fluorescent magnetic nanoparticles. Under the effect of an *in vitro* magnetic field, using a thermal imager, it was also detected that the local temperature of the tumor reached above 45 °C, while the temperature of important organs remained below 38 °C. Therefore, the implementation of magnetic thermotherapy on tumors *in vivo* using an applied magnetic field provides positive therapeutic results [118, 119]. They next synthesized CXCR4 antibody-modified gold nanorods and co-incubated them with human iPS cells, observing that CXCR4 antibody-modified gold nanorods entered human iPS cells efficiently. In a

tumor-bearing mouse model, iPS cells loaded with CXCR4-modified gold nanorods were injected through the tail vein, and it was observed that the iPS cells were able to target the tumor site, and the photothermal treatment of the tumor could be achieved based on the light-absorbing and heat-producing properties of the gold nanorods [120]. Cancer stem cells are an important cause of tumor metastasis and recurrence. They prepared a CD44 antibody-coupled gold nanostar probe, which successfully achieved targeted photoacoustic imaging, CT imaging, and photothermal therapy for gastric cancer stem cells, significantly inhibiting the growth of cancer stem cells and having clinical translational value [49]. The current series of progress shows that stem cell nanotechnology has broad application prospects in stem cell research and development, and it is a critical field of precision medicine.

Another crucial area of precision medicine research is the research and development of nano-targeted drugs. Targeted drugs are designed for the tumor-specific targets, enabling targeted tumor imaging and treatment while also observing the therapeutic effect. For example, Conde et al. prepared 90 nm gold nanoparticles coupled with Raman reporter molecules. The gold nanoparticles were PEG-modified and combined with the antibody-drug conjugate Cetuximab, and the prepared antibody drug SERS gold nanoantennas achieved specific binding to EGFR of lung cancer cells, blocked the EGF protein from reaching the cancer cells and inhibited the signalling cascade, consequently stopping proliferation and survival of cancer cells, while achieving Raman spectroscopic imaging and boundary determination of tumor tissue boundaries [121]. Conde et al. reported that glucose-coupled gold nanoparticles were used for near-infrared fluorescence imaging and targeted therapy of tumors, and realized *in vitro* and *in vivo* siRNA delivery therapy using multifunctional gold nanoparticle probes [122], in which gold nanoprisms were used for targeted photoacoustic imaging of gastrointestinal tumors and siRNA/RGD-coupled gold nanoparticles were used for targeted imaging and therapy of lung cancer, inducing an inflammation-like response and immune response, presenting a low-dose siRNA therapeutic effect. In addition, folic acid-coupled gold nanorods could be used for enhanced radiation therapy under fluorescence and CT imaging; BRCAA1 antibody-coupled gold nanoprisms could be used for targeted photoacoustic imaging and photothermal therapy of gastric cancer [123]; folic acid-coupled ce6-modified gold nanoclusters could be used for targeted imaging photodynamic and photothermal therapy of gastric cancer; photosensitizer-coupled gold nanovesicles could be used for photothermal and photodynamic therapy under imaging. Nano-responsive drugs designed for tumor microenvironments (such as pH, ROS, hypoxia, etc.) can enable targeted imaging as well as chemical and photodynamic therapy of tumors [124, 125].

Improving the solubility of insoluble drugs, promoting drug absorption, and enhancing drug bioavailability are all pressing issues in the field of pharmacy. The combination of nanotechnology and pharmacy is expected to accelerate the resolution of this problem, which is also an important research direction in the field of nanoprecision medicine. The application advantages of nanotechnology are increasingly emerging: nanosizing greatly reduces the particle size of drugs and greatly increases the surface area; insoluble drugs can form a higher local concentration in nanocarriers, while also improving their dispersion in water to form a stable colloidal solution; the adhesion of drug is enhanced, and the residence time at the absorption site is prolonged; nano drug delivery systems can improve the membrane permeability and stability of drugs, which is conducive to improving the bioavailability of the drug, especially for drugs of class II (low solubility, high permeability) and class IV (low solubility, low permeability) of the biopharmacological classification system (BCS), this technology is increasingly favored by several research institutions and pharmaceutical companies both at home and abroad [126]. For example, in 2010, researchers from the Department of Cell Research and Immunology at Tel Aviv University in Israel and the Center for Nanoscience and Nanotechnology achieved a breakthrough in drug delivery. They developed a nanocarrier that can deliver chemotherapy drugs directly to tumor cells without interacting with normal cells. This not only enhances chemotherapy efficacy, but also reduces the toxic side effects of chemotherapy drugs, improves the adaptability and therapeutic effect of patients.

New fields of nanoprecision medicine will continue to emerge and improve. With the development of nanobiotechnology, researchers will not only imitate

the assembly of existing molecules in nature, but also achieve deeper and purposeful control of molecular structures. One of the most important difficulties of nanotechnology is the inability to actually see how the research object behaves. For example, viruses are smaller than the wavelength of light, and their biological structures cannot be seen with standard light microscopy, and are also difficult to observe with other imaging techniques. A multidisciplinary collaborative research team established at the University of California, Los Angeles, used cryo-electron microscopy to reveal the precise atomically resolved three-dimensional structure of adenoviruses and the interactions of their protein networks, and that the viruses can be modified to enable them to deliver drugs to diseased sites, this discovery provides critical information for researchers around the world attempting to modify adenoviruses for use in vaccines and cancer gene therapy [127].

With the continuous progress in chemistry, physics, biology, materials science, and other fields, some nanoscale structures can be compatible with living cells and proteins, and can be tailored to manufacture nanomedicines and nanodevices for disease diagnosis and prevention, which is an important translational component in nanoprecision medicine. The report "Nanobiotechnology: Applications and Global Markets" pointed out that nanobiotechnologies may be commercialized in the next five years include: drug delivery, diagnostics, R&D tools, microbicides and DNA sequencing. The nanobiotechnology product market will grow at a compound annual growth rate of 9%, and the market size in 2015 has reached $29.7 billion. The market for medical applications of nanobiotechnology will grow at a compound annual growth rate of 8.7%, to $34.2 billion in 2017. In the R&D tools market, DNA sequencing emerges as an emerging market opportunity for nanotechnology. In 2010, this segment of the market was $63 million, and in 2017 it has exceed $350 million, with an annual growth rate of 37%.

In the near future, nanobiotechnology will be widely used in clinical medical applications. Nano drug carriers applied to targeted treatment of malignant tumors will become a new method of diagnosis and treatment, and nano gene carriers will advance the clinical application of gene therapy. Nano-probe diagnostic technology and nano-magnetic bead cell separation technology are widely used in clinical and biotechnology product development, nanobiomaterials as human implants and applied in tissue engineering will solve many drawbacks of traditional materials in clinical applications, and nanotechnology transformation of traditional drugs and traditional Chinese medicine processing technology will also improve the therapeutic effect of traditional Chinese medicine to a large extent. The combination of disease diagnosis technology and treatment technology is the development direction in the future, and the development of nanobiotechnology will help realize the integration of diagnosis and treatment technology as soon as possible [128, 129]. Meanwhile, the use of nanobiotechnology can also combine chemotherapy, gene therapy, radiotherapy, cell therapy and other technologies to improve the effect of tumor treatment, and how to realize the integration of these technologies is the future development direction.

Challenges of integrating nano-oncology

The integration of nano-oncology, akin to the burgeoning discipline, presents numerous hurdles. It is widely acknowledged that the human body embodies an intricate framework encompassing neural networks dispersed across its entirety, and the harmonious interplay among molecules, cells, tissues, and organs sustains optimal physiological operations [130–132]. Achieving the desired functionalities of nanomaterials, nanoprobes, nanomedicines, and nanodevices within the human body presents a formidable challenge, as their introduction necessitates meticulous exploration to ascertain their impact on the intricate operations of other organs, cells, and tissues. Extensive research efforts are required to substantiate these claims. Numerous investigations have provided evidence indicating that the delivery of nanomaterials and devices to the intended therapeutic site remains predominantly successful [133–138], while only a minority traverse towards alternative sites. Nevertheless, the impact of these entities on the functionality of non-targeted sites remains enigmatic, necessitating a plethora of studies to ascertain their potential harm or benign nature, discern the nature of their effects (beneficial or adverse), and resolve the ongoing debate surrounding their *in vivo* safety. Moreover, the absence of systematic experimental and theoretical

methodologies hinders our ability to quantitatively investigate the structural, mechanical, and deformation properties of nanomaterials and devices, among other subjects. Consequently, the establishment of robust theoretical models presents substantial challenges in this field.

The integration of nano-oncology necessitates the utilization of a considerable array of precision-based nanomaterials, nanoprobes, nanomedicines, nanodevices, smart instruments. Nanomaterials and nanomachines for vitro diagnostic have no effect on the human body, but it is also a great challenge to achieve precise manufacturing and large-scale preparation of these nanoprobes and nanomachines that enter the human body. molecular printing [139], self-assembly [140], soft etching [141,142], and atomic force manipulation [143] had been successful applied in the preparation of nanomaterials, but achieving precise assembly of nano-system remains a formidable challenge. Given the novelty and high degree of innovation associated with nanomaterials, nanoprobes, and nanodevices, the establishment of a universally acknowledged manufacturing standard and a scientifically sound evaluation framework presents a formidable challenge.

The perspectives of integrating nano-oncology

The integration of nano-oncology, capitalizing on the inherent advantages of nanotechnology, holds the potential to address the scientific and technical challenges encountered in tumor diagnosis and achieve precision diagnosis and treatment with utmost accuracy. Particularly, micro and nano manufacturing technologies drive diagnostic and therapeutic advancements towards intelligent, automated, miniaturized, and personalized approaches. Novel nanomaterials such as grapheme [144], carbon nanotubes [145], quantum dots [146,147], and gold nanoclusters [148,149] propel the development of biomedical materials towards superior performance, enhanced biocompatibility, and the induction of differentiated growth. The design and fabrication of nanoprobes facilitate molecular imaging advancements, while the fusion of nanotechnology with traditional Chinese medicine significantly enhances the water solubility, safety, and efficacy of such remedies [150]. Furthermore, the evolution of DNA nanotechnology [151] and RNA nanotechnology [152] enables the creation of nanotechnology with chemical drugs addresses challenges related to solubility, while the convergence of nanotechnology and sequencing technology fosters the emergence of a new generation of nano-sequencing technologies. This, in turn, fuels the development of core nano-sequencing instruments, frontier technologies, and self-reliant research and innovation capabilities, ultimately strengthening independent core competitiveness and alleviating dependence on foreign imports.

In summary, the convergence of nanotechnology and integrated medical oncology, culminating in the field of integrated nano-oncology, exhibits remarkable technical merits and holds immense potential for practical applications. As integrated nano-oncology continues to advance steadily, it is poised to generate substantial economic and societal dividends, reshaping our world and enhancing the quality of human life.

CRediT Author Statement

Fuhua Yang, Daxiang Cui: conceptualization, methodology, supervision, review structure conception. **Jinlei Jiang, Xinyuan Cui, Yixin Huang, Dongmei Yan:** investigation, visualization, data curation, and writing-original draft preparation. **Bensong Wang, Ziyang Yang, Mingrui Chen, Junhao Wang, Yuna Zhang:** formal analysis, supervision, and validation. **Guan Liu, Cheng Zhou, Shengsheng Cui, Jian Ni:** writing-reviewing, and editing.

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Conflict of Interests

The authors declare that they have no conflict of interest.

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