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Activatable Molecular Agents for Cancer Theranostics

Jianjian Zhang,*a Lulu Ning,b Jiaguo Huang,c Chi Zhang and Kanyi Pu*c

Theranostics that integrates diagnosis and treatment modalities has attracted great attention due to its abilities of personalized therapy and real-time monitoring of therapeutic outcome. Such a theranostic paradigm requires agents to simultaneously possess the capabilities of targeting, imaging, and treatment. Activatable molecular agents (AMAs) are promising for cancer theranostics, as they show higher signal-to-noise ratio (SNR), real-time detection of cancer-associated biomarkers, lower normal tissue toxicity, and higher therapeutic effect. This perspective summarizes the recent advancements of AMAs, which include imaging-guided chemotherapy, imaging-guided photodynamic therapy, and imaging-guided photothermal therapy. The molecular design principles, theranostic mechanisms, and biomedical applications of AMAs are described, followed by the discussion of potential challenges of AMAs in cancer theranostics.

1. Introduction

Cancer, also known as malignant tumor, is one of the major diseases that threaten people’s lives globally.1 Traditional tumor treatments mainly include surgical resection, chemotherapy, and radiotherapy. However, the unmarked remaining cancer cells after surgical resection are easy to metastasize, leading to postoperative recurrence. Moreover, chemotherapy and radiotherapy show a significant killing effect both on tumor and normal tissues. Besides, tumor cells are prone to develop resistance to chemotherapy and radiotherapy.2, 3 Therefore, the combination of timely diagnosis of cancer and effective treatment methods has become a research focus.

Theranostics, a new biomedical technology that combines the diagnosis with therapy, shows great potential in personalized cancer medicine, including real-time monitoring of the treatment process and reflecting the feedback of therapeutic effect. So far, great efforts have been devoted to the development of theranostic agents.4-8 There are two main kinds of strategies. One is to use a chemical synthesis method. Various anticancer molecules, imaging agents and cancer-targeting molecules are covalently conjugated together through chemical reactions, affording the macromolecular agents capable of tumor targeting, imaging, and therapy. The other is nanoparticle encapsulation. Imaging agents and anticancer molecules are encapsulated in amphiphilic coatings to form nanoparticles, which can be delivered to the solid tumor through specific targeting or enhanced permeability and retention (EPR) effect. Numerous theranostics systems combining different imaging techniques (e.g., magnetic resonance imaging (MRI), positron emission tomography (PET), fluorescence imaging (FLI), and photoacoustic imaging (PAI)) with diverse therapeutic methods (including chemotherapy (CHT), photodynamic therapy (PDT), photothermal therapy (PTT), and gene therapy (GT)) through one (imaging)-to-one (therapy), many-to-one, one-to-many, and many-to-many types have been developed.9-32 Since different modalities can make up for each other’s limitation, offer improved imaging quality and/or therapeutic efficacy, and minimize the probability of over-medication, the development of single molecular probe integrated with multi-modality approaches showed great potential in the treatments of cancers.

However, most theranostic agents employ “always-on” mode for both diagnosis and therapeutic intervention, leading to limited signal-to-noise ratio (SNR) in the disease sites and notable side effects to normal tissues.33-37 In contrast, activatable theranostic agents capable of intrinsic signal changes with simultaneous initiation of therapeutic action upon detection of biomarkers (from the “off” state to the “on” state) demonstrate potentials of higher SNR, lower limit of detection (LOD), real-time detection of biomarkers, lower normal tissue toxicity, and higher drug bioavailability. However, such an approach is challenging as it requires recognition of reactions that are precisely induced by a single species. Hence, activatable theranostic agents have been far less developed.

Many multi-modality materials have been exploited as theranostic agents, such as upconversion nanoparticles (NPs),38 carbon nanotubes,39 gold nanoclusters,40 reduced graphene oxide,41 magnetic NPs,42 quantum dots,43 semiconducting polymer nanoparticles (SPN),23, 28, 44-49 and small molecular dyes.9, 50-52 Each has its own advantages and shortcomings. For instance, inorganic nanomaterials possess excellent properties of optical tunability and phototherapeutic ability, but their toxicity in clinical application still needs to be solved urgently.53, 54 Molecular agents have long been a research focus in life sciences and biomedicine owing to their significant merits, such as simple and controllable molecular
structure, easy production in large scale, better biocompatibility, and easy metabolism in living organisms. Hence, distinct breakthroughs and evolutions of theranostic based on AMAs have been achieved during past years and could noteworthy accelerate the development of cancer theranostics.

**Chemotheranostics**

- a. Chemotherapy with extra signal group
- b. Chemotherapy without extra signal group

**Phototheranostics**

- c. Photodynamic therapy (PDT)
- d. Photothermal therapy (PTT)

The design strategies of molecular-based theranostic agents with improved therapeutic effects and minimized side effects have been recently reviewed. Some of which have been already used in clinical for PDT such as porphyrin, 5-aminolevulimic acid and their derivatives. Herein, this perspective mainly describes the latest research achievements of AMAs. In the following, we summarize and discuss different therapeutic modalities that have been attained by AMAs (Figure 1). Design principles, therapeutic mechanisms, and biological applications of these theranostic agents are substantially illustrated. Moreover, discussion regarding the potential challenges of AMAs is also provided.

2. Chemotheranostics

Chemotherapy as a dominant treatment modality for various cancers utilizes chemical drugs such as doxorubicin (DOX), camptothecin (CPT), etoposide, 5-crizotinib, 7-ethyl-10-hydroxycamptothecin (SN-38), gemcitabine, and paclitaxel to prevent the anabolism of nucleic acids, DNA replication, spindle formation, and protein synthesis. Such inhibition retards the growth and reproduction of tumor cells, finally destroying cancer cells. Although these current clinical chemotherapeutic agents demonstrate high efficiency in anticancer progression, it has serious drawbacks such as poor selectivity towards cancer cells, low drug bioavailability, and high systemic toxicity. To solve these problems, activatable theranostic agents that integrate targeted molecular optical reporters with chemotherapy drugs are developed by some research groups. When interacting with various tumor-associated stimulus (e.g., high reactive oxygen species (ROS) level, hypoxia, highly expressed enzyme, acidity, and reducibility), the activatable theranostic agents can precisely kill cancer cells. Meanwhile, the easy-to-monitor signals of the optical reporter also change from “off” to “on”. Furthermore, a series of molecular-based theranostic agents with improved therapeutic effects whereas minimized side effects have been recently developed.

2.1. Fluorescence imaging-guided CHT

Fluorescence imaging technique has become an indispensable tool in biological research due to the versatile emission spectra, non-invasiveness, high temporal and spatial resolution, fast-scanning speed and real-time monitoring. Encouraged by the high performance of molecular probes in both FLI and CHT, AMAs have been widely used in applications of FLI-guided CHT. The platforms can be divided into three types: (i) drugs connected with a fluorophore through a linker; (ii) therapeutic agents with fluorescent features connected with the targeting group through a linker; (iii) both drugs and fluorophore are encapsulated in polymers or mesoporous materials.

For instance, based on a multicomponent (fluorophore, drug, linker, and targeting group) synthesis strategy, Kim et al. constructed a theranostic agent, RGD peptide-appended naphthalimide pro-camptothecin (RGD-NAP-CPT) for imaging-guided chemotherapy (Figure 2a). RGD-NAP-CPT was a multifunctional molecule composed of an RGD cyclic peptide as a particularly effective cancer-targeting unit, a naphthalimide fragment as a fluorophore, a disulfide bond as a glutathione (GSH) cleavable linker, and camptothecin (CPT) as an antitumor inhibitor of topoisomerase I. The prodrug agent was endocytosed into the targeted cancer cells through the interaction between RGD and αβ3 integrin receptor. Upon reaction with GSH that was relatively abundant in tumor cells, disulfide cleavage occurred, leading to the release of the free cytotoxic CPT in the endoplasmic reticulum, along with naphthalimide that emitted an intense red-shifted fluorescence signal at 535 nm. The CPT, in turn, diffused into the nucleus of the cancer cells, inhibiting the activity of topoisomerase I and achieving the anti-tumor effect (Figure 2b). These results demonstrated that RGD-NAP-CPT could act as a theranostic agent to provide FLI-guided CHT.

To illustrate the universality of this strategy, Kim and coworkers performed a cancer-targeting theranostic smart drug delivery system based on a gemcitabine-coumarin-biotin (GMC-COU-BT) conjugate (Figure 2c). GMC-COU-BT was composed of a cancer-targeting ligand biotin, a thiol-specific cleavable disulfide bond, an activatable fluorescent reporter coumarin moiety in response to disulfide bond cleavage, and gemcitabine (GMC) as a model active drug. By tracking the green emission of the coumarin molecules, lysosome trapping of GMC followed with drug release to the cell cytoplasm (Figure 2d), and the distribution of the drug was revealed. Besides, the therapeutic effect of this theranostic agent could be easily monitored at the subcellular level by two-photon fluorescence imaging.
In the development of theranostic agents, the utmost challenge lies in the low specificity and high systemic toxicity. Targeting the theranostic agents to cancer cells helps to improve therapeutic efficiency. However, this visualization of targeted drug delivery requires additional fluorescent labelling, which may change the route of drug molecules. Hence, there is an urgent need to design therapeutic agents with intrinsic fluorescence for guiding and displaying therapeutic effects. Shin et al.\textsuperscript{73} developed a dual-targeting therapeutic agent (Oct-Dox) via conjugation of a synthetic ligand (octreotide) of the cancer-selective somatostatin receptor, a peptide substrate that can be cleaved by cathepsin B overexpressed in cancer cells, to an anticancer agent with intrinsic fluorescence (Figure 3a). The theranostic agent was employed to image and kill cancer cells expressing both somatostatin receptors and cathepsin B. In contrast, normal cells with low expression of somatostatin receptors and cathepsin B were not affected. The new dual-targeting theranostic agent has been proved effective in FLI-guided cancer CHT.

Following the idea, Perez and coworkers\textsuperscript{66} developed an activatable targeted small molecular-based theranostic agent Doxo-S-S-Fol. Such a nanosystem composed of folic acid receptor that is highly expressed in various solid tumors, a disulfide linker cleavable by glutathione (GSH) or thioredoxin (Trx) that are relatively abundant in tumor cells, and DOX, a fluorescent antitumor drug (Figure 3b, c). Doxo-S-S-Fol was activated via disulfide-cleavage in the presence of intracellular reduced GSH, showing enhanced fluorescence and cellular toxicity only in folate-receptor positive cells and exhibiting extremely low toxicity to cells that do not express the receptor. The results presented in this work showed the potential to guide the development of AMAs, thus establishing imaging of drug distribution, drug activation, and therapeutic efficacy.

**Figure 2.** (a) Structure of theranostic agent RGD-NAP-CPT. (b) Schematic mode of action of RGD-NAP-CPT in cancer cells. RGD-NAP-CPT is selectively internalized in the cells through receptor-mediated endocytosis. Fluorescence emission and cytotoxicity of the prodrug are activated due to cleavage of the disulfide bond by GSH. Adapted from Ref. \[66\]. Copyright 2012 American Chemical Society. (c) Structure of theranostic agent GMC-COU-BT. (d) Confocal microscopic images of colocalized experiment in A549 cells. Adapted from Ref. \[68\]. Copyright © 2013 American Chemical Society.
Because of major limitations such as low biological availability, multi-drug resistance, and high systemic toxicity, traditional chemotherapeutic agents are severely restricted in clinical applications. Nanoparticles used as drug carriers provide new opportunities to increase solubility, prolong bioavailability, reduce side effects, target drug delivery, and combat multi-drug resistance for cancer therapy. Following the idea, a theranostic nanosystem (RA-S-S-Cy@PLGA NPs) with redox dual-activatable and O$_2$-evolving has been developed by Tan and coworkers. A natural homodicyclohexapeptides deoxybouvardin (RA-V) as the active antitumor agent, a near-infrared (NIR) fluorescence dye Cy5.5 as a fluorophore to monitor the activation of RA-V, and a disulfide bond as a thiol-specific cleavable linker were covalently bonded to form the theranostic agent (RA-S-S-Cy). And RA-S-S-Cy@PLGA NPs were constructed by loading a theranostic agent RA-S-S-Cy and an O$_2$-generating agent catalase into D,L-lactic-co-glycolic acid (PLGA) nanoparticles that were conjugated with a tumor-targeting cyclic pentapeptide c(RGDfK) (Figure 4a). The theranostic nano agent modified with c(RGDfK) was endocytosed into the targeted cancer cells via αvβ3 integrin-mediated internalization. Later, a high level of intracellular H$_2$O$_2$ in αvβ3 integrin-rich cancer cells penetrated the RA-S-S-Cy@PLGA NPs, leading to a huge amount of oxygen production. The PLGA shell wall was instantly disrupted by O$_2$ bubbles, and leading to the release of RA-S-S-Cy. As such, RA-V was released in a cell-specific and redox dual-activatable manner, leading to cellular apoptosis and enhanced NIR fluorescence (Figure 4b). Moreover, RA-S-S-Cy@PLGA NPs were successfully applied to monitor drug release and chemotherapeutic efficacy in vivo. As a result, the nano platform provides a potential tool for a profound understanding of drug release mechanisms and chemotherapy process.
NPs upon exposure to GSH. Adapted from Ref. [75]. Copyright © Ivyspring International Publisher.

2.2. Photoacoustic imaging (PAI)-guided chemotherapy (CTH)

PAI, also known as optoacoustic imaging, is currently one of the burgeoning biomedical imaging techniques.\(^{79,80}\) In comparison with traditional optical imaging which suffers from shallow tissue penetration depth,\(^{81}\) molecular PAI that combines NIR laser excitation with ultrasonic detection can provide high spatial resolution mapping in deep tissues (>12 cm) while retaining high contrast of optical imaging to guide accurate theranostics.\(^{80,82,83}\) Although activatable photoacoustic probes have the capability of imaging cancer-specific biomarkers, the effective drug delivery and drug release monitoring are hampered due to the limitations of their molecular structures. Hence, for theranostic agents, the contrast agents with high molar absorbance (extinction) coefficient and low non-radiative quantum yield in the near-infrared region are urgently needed.\(^{84}\)

Recently, a multifunctional nano theranostics prepared by nanoparticle encapsulation for real-time ratiometric PA imaging of tumor acidic pH and monitoring of drug release in living mice has been developed by Chen et al.\(^{85}\) The theranostic platform (THPDINs) was first constructed by self-assembly of the acid-responsive amine-substituted PDI, a pH irrelevant IR825 dye, and anti-cancer drug DOX (Figure 5a). The typical small semiconducting molecule of PDI showed a blueshift of its absorption from 680 nm to 530 nm in an acidic environment (Figure 5b), which caused a reduction in photoacoustic signals of PDI at 680 nm. Also, in the mild acidic tumor microenvironment, the THPDINs was induced to a loosened nanostructure that could accelerate the release of the encapsulated DOX together with PA signals weakened at 680 nm. Meanwhile, acting as an internal reference, IR825 was still retained the same including its chemical structure and characteristic PA signal at 825 nm (Figure 5c). Moreover, the antitumor efficacy of the THPDINs was also evaluated in U87MG tumor mice (Figure 5d). The mice treated with other contrast reagents such as PBS, HPDINs, and DOX-loaded APDINs, exhibited rapid tumor growth, and that treated with free DOX showed slower tumor growth. THPDINs could effectively inhibit tumor growth. Besides, no remarkable body weight loss was observed. These results indicated that the theranostic platform of THPDINs showed high anti-cancer effects, good biocompatibility, and little side effects. This strategy will provide a reference for the development of smart activatable theranostic nanoplatforms and will greatly promote the application of PA-guided chemotherapy in biology and medicine.

![Figure 5](image_url)

**Figure 5.** (a) Schematic illustration of the sensing and drug releasing mechanism of THPDINs. The THPDINs is self-assembly with a pH-sensitive HPDI (green), an anticancer drug of DOX (blue), and a pH-inert IR825 (gold). (b) UV–vis absorption spectra of THPDINs. (c) In vivo PA imaging of tumor pH. Representative PA images of a subcutaneous U87MG tumor in a nude mouse after intravenous administration of the THPDINs at post-injection time 0, 4, 24, and 48 h. (d) Representative photographs of tumor-bearing mice resected U87MG tumors in different treatment groups on day 18. Adapted from Ref. [81]. Copyright © Ivyspring International Publisher.

3. Phototheranostics

Phototherapy,\(^{8}\) mainly including PDT and PTT, has obtained considerable attention owing to its features with minimal invasiveness, rapid treatment, high temporal-spatial controllability and low systemic toxicity for cancer therapy. In general, both PDT and PTT need the accumulation of phototherapeutic agents in the tumor site and subsequently pinpoint light irradiation with the corresponding wavelength. PDT mainly consists of three indispensable elements: photosensitizer, excitation light, and oxygen...
molecules in tumor tissues. Upon the light irradiation with specific wavelengths, the photosensitizers convert molecular oxygen into cytotoxic ROS (such as $\text{O}_2^-$, $\text{OH}$, and $\text{O}_2$) after absorbing NIR photons, which enable to destroy cancer cells through oxidative stress and therefore induce apoptosis. Activatable PDT could enhance the selectivity of cancer therapy through introducing modified photosensitizers with a bio-responsive element, which was unable to produce ROS even upon irradiation. Only upon activation to ultimately restore the native photosensitizers can produce cytotoxic ROS under light irradiation. As a result, global irradiation can cause only cancer cells to die leaving healthy cells unharmed, thereby minimizing side effects. Also, activatable PTT employs phototherapeutic agents with the photothermal conversion ability only when stimulated by tumor markers, which transfer external light energy into heat, causing the temperature increase at the tumor site, and destroying the function of cancer cells, hence achieving the purpose of antitumor treatment. Benefiting from high tumor specificity, short irradiation time, rapid treatment processes, repeatable operability, no drug resistance, and good therapeutic effect, activatable PDT and PTT have the potential to be independent cancer treatment methods, respectively, and numerous novel theranostic agents combine optical imaging with phototherapy have recently been developed.

### 3.1. Fluorescence imaging-guided PDT

In the aspect of FLI-guided therapy, the selection of high specificity of activatable theranostic agents with excellent optical properties is a great challenge for PDT in cancer treatment. Recently, a special kind of photosensitizers with aggregation-induced emission (AIE) character has been successfully developed for PDT, because these AIEgens can emit signals in the whole visible range, even in the NIR region, which have been demonstrated to be highly efficient ROS generators as aggregates. Taking advantage of these mentioned features, a novel bio-probe TPETF-NQ-cRGD has been developed by Liu and coworkers. The probe, composed of a tetraphenylethylene moiety with AIE properties as an imaging agent, a biothiols cleavable 2,4-dinitrobenzenesulfonyl (NQ) as a quencher moiety, and a cyclic arginine–glycine–aspartic acid (cRGD) tripeptide as a targeting group of $\alpha_v\beta_3$ integrin overexpressed in cancer cells, can specifically recognize and ablate cancer cells (Figure 6a,b). The fluorescence and photosensitizing activity of TPETF-NQ-cRGD was quenched in the aggregated state. After TPETF-NQ-cRGD enters the $\alpha_v\beta_3$ integrin-GSH dual-overexpressed cancer cells by receptor mediated endocytosis, followed by intracellular GSH activation. (b) The probe keeps an "off" state which is non-fluorescent with almost no ROS generation upon light irradiation, but the fluorescence and ROS generation can be turned "on" after receptor mediated endocytosis, followed by intracellular GSH activation. Confocal laser scanning microscope images of MDA-MB-231 cells pre-incubated with cRGD (E) or BSO (F) and further incubated with TPETF-NQ-cRGD (10 mM) for 1 h (A), 2 h (B), 4 h (C), and 6 h (D) (Figure 6b). The probe was quickly killed by $\text{O}_2$ which was rapidly produced by TPETF. Besides, the AA linker was efficiently cleaved by $\text{O}_2$, leading to an obvious green fluorescence from rhodol. This probe design thus represented an advanced strategy for real-time in situ monitoring of ROS production during PDT through self-tracking.

**Figure 6.** (a) Schematic illustration of probe TPETF-NQ-cRGD activated by glutathione (GSH) to release the photosensitizer. (b) The probe keeps an “off” state which is non-fluorescent with almost no ROS generation upon light irradiation, but the fluorescence and ROS generation can be turned “on” after receptor mediated endocytosis, followed by intracellular GSH activation. (c) Confocal laser scanning microscope images of MDA-MB-231 cells pre-incubated with cRGD (E) or BSO (F) and further incubated with TPETF-NQ-cRGD (10 mM) for 1 h (A), 2 h (B), 4 h (C), and 6 h (D). (d) Cytotoxicity of the probe to MDA-MB-231 cells with different times of light irradiation. Adapted from Ref. [86]. Copyright ©2016 Royal Society of Chemistry.

Later on, a theranostic agent TPETF-AA-Rho-cRGD (Figure 7) containing cRGD, $\text{O}_2^-$-responsive green-emissive fluorogenic rhodol moiety, $\text{O}_2$-cleavable linker aminoacrylate (AA), and red-emissive AIE-active unit (TPETF) was successfully designed by the same group. The probe TPETF-AA-Rho-cRGD can emit red fluorescence from the TPETF moiety in aqueous solution, which endows it the ability to track itself. The cRGD with targeting function made TPETF-AA-Rho-cRGD specifically target $\alpha_v\beta_3$-overexpressing cancer cells. Upon image-guided light irradiation, the DA-MB-231 cancer cells were quickly killed by $\text{O}_2$ which was rapidly produced by TPETF. Besides, the AA linker was efficiently cleaved by $\text{O}_2^-$, leading to an obvious green fluorescence from rhodol. This probe design thus represented an advanced strategy for real-time in situ monitoring of ROS production during PDT through self-tracking.
3.2. Fluorescence and Photoacoustic imaging-guided PDT

Since each imaging modality (FL, PA, PET, MRI, etc.) has its advantages and limitations, dual-mode or multi-mode imaging techniques are often used to collect complementary information to verify the diagnosis.94, 95 As we all know, fluorescence imaging possesses high sensitivity and outstanding resolution, but it suffers from low penetration depth. While PA can provide desirable tissue penetration depth. As such, a diagnostic probe with FL and PA dual modalities could provide much more precise information, which shows great potential for biological applications.82, 96-98

Cai et al.99 have developed a first small-molecule “Four in One” theranostic probe RhoSSCy that integrates tumor targeting, pH/thiols detection, near-infrared fluorescence (NIRF)/PA dual-modal imaging, and PDT therapy (Figure 8a, b). The probe was constructed by heptamethine cyanine IR765 (Cy) and carboxyrhodamines (Rho) via the cystamine linker. In the probe, the reducible disulfide and amino-group are acted as thiols recognition group and pH tunable sensor, respectively. In vitro experiments demonstrated that the probe could quantitatively analyze and image intracellular thiols and pH gradient with high sensitivity. Moreover, the probe exhibited splendid tumor-targeted and accumulation, which was verified by the near-infrared fluorescence (NIRF) and photoacoustic (PA) dual-modal imaging of tumors (Figure 8c, d).

Significantly, RhoSSCy could generate ROS in tumors upon dual-mode imaging-guided light irradiation and achieve robust antitumor activity through PDT. Such AMAs have the great potential in precision detection and efficient therapy of tumors.

3.3. Chemiluminescence imaging-guided PDT

Chemiluminescence, a phenomenon of light radiation accompanied by chemical reactions, has been increasingly recognized as a powerful tool for disease diagnosis and biological analysis.100-102 Compared to traditional fluorescence imaging, CL has the advantage of high sensitivity, low phototoxicity, and high signal-to-noise ratio due to the removal of the external excitation source. Peroxalate derivatives worked as light-emitting functionalities have been widely explored for chemiluminescence. It can be oxidized by hydrogen peroxide (H₂O₂) to form a high-energy and unstable dioxetanedione, which is an active chemiluminescent substrate that spontaneously undergoes decomposition to emit photons and the energy is transferred to a nearby fluorescent dye, leading to a particular optical signal. Based on this, chemiluminescence can be worked as an appropriate imaging method for specific tumor imaging due to a higher amount of H₂O₂ in solid tumors than that in normal tissues,103 and it is widely used as a guiding technique in photodynamic therapy.104, 105

Encouraged by the great properties of chemiluminescence, Liu et al.105 developed a novel therapeutic agent that based on CL-guided PDT for accurate diagnosis and treatment of cancers. The therapeutic agent C-TBD nanoparticles (C-TBD NPs) were composed of Bis[2,4,5-trichloro-6-(pentyloxycarbonyl)phenyl] oxalate (CPPO) as the chemical excitation source and TBD as the high efficiency...
photosensitizer with the capability of FR/NIR emission and ROS production, soybean oil as a retarder, and pluronic F127 as the encapsulating agent (Figure 9a). In this nanosystem, a high-energy 1,2-dioxetanedione intermediate, formed from CPPO under the action of H$_2$O$_2$, can excite TBD through a chemically initiated electron exchange luminescence (CIEEL) process. Finally, the excited TBD can generate luminescence and react with oxygen to generate $\mathrm{^{1}O_2}$ without an extra light source, which provides a novel strategy in tumor imaging and treatment. The in vivo experiments demonstrated that C-TBD NPs could specifically track tumors through chemiluminescence imaging. In contrast, fluorescent signals in both the tumor and reticuloendothelial system (RES) organs (such as the liver) were observed (Figure 9b, c). Besides, compared with the other control groups, the tumors of mice treated with both C-TBD NPs and an H$_2$O$_2$ enhancer agent β-phenylethyl isothiocyanate (FEITC) exhibited the most significant growth inhibition (Figure 9d), verifying the effectiveness of the combination therapy. This work presents a novel strategy for CLI-guided PDT, which can motivate more researches for tumor diagnosis and treatment.

Figure 9. (a) The preparation of C-TBD NPs and illustration of the principle for chemiluminescence and $\mathrm{^{1}O_2}$ generation of C-TBD NPs in the presence of H$_2$O$_2$. (b) Schematic illustration of activated C-TBD NPs to image tumors within the H$_2$O$_2$-enriched tumor microenvironment. (c) Time-dependent in vivo chemiluminescence (top) and fluorescence (bottom) images of mice injected C-TBD NPs (1 mg/mL based on C-TBD, 100 mL per mouse) over 5 h periods. Tumor regions are marked with yellow circles. (d) Tumor growth curves with different therapies. Adapted from Ref. [99]. Copyright © 2017 Elsevier.

3.4. Fluorescence imaging-guided CHT and PDT

Materials based on a single therapy mode may not be able to match the complex biological environment effectively due to their limited functions. Therefore, the design of dual-mode or multi-mode therapy materials have become a new development trend. Imaging-guided chemo-photodynamic dual-mode therapy is a novel modality of cancer theranostics, which can improve therapeutic efficiency with minimized side effects and avoid the burden of multiple injections of different therapeutic agents in clinical applications. Liu and coworkers developed a kind of theranostic agent (Co-NPs) that was constructed by self-assembly of paclitaxel (PTX, an anti-tumor drug for ovarian and breast cancer) dimer and two-photo photosensitizer (Figure 10a). Upon short light irradiation, an increasing bright fluorescence signal from Co-NPs in lysosome was observed, which was ascribed to the photochemical internalization (PCI) effect, leading to an enhanced cellular uptake. Then, Co-NPs escaped from the lysosome into the cytoplasm after 808 nm laser irradiation, which was proved by the lysosome colocalization experiment. Synchronously, $\mathrm{^{1}O_2}$ was generated by the photoactivation of the photosensitizer 2PE-PS in Co-NPs, thus killing the cells by PDT. In the presence of GSH, the disulfide bond in PTX dimer was cracked and the paclitaxel monomer was released. The free PTX diffused to the cytosol for chemotherapy (Figure 10b). Hence, the combination CHT-PDT pathway based on fluorescence molecular theranostic agents showed great potential for cancer theranostics.

Figure 10. (a) Preparation of nanoparticles. (b) Schematic illustration of the synergistic CHT and PDT. Adapted from Ref. [20]. Copyright © 2019 Elsevier.
3.5. Fluorescence and Photoacoustic imaging-guided PTT

PTT that requires efficient conversion of photon energy into heat, is consistent with the principle of PA imaging.40,111 Significantly, PTT has advantages over PDT in terms of cancer therapy, owing to the therapeutic efficiency of PTT frequently higher than PDT, and PTT can work well in the hypoxic tumor environment. Hence, the combination of fluorescent/photoacoustic imaging and photothermal therapy proved to be an eminent modality in cancer theranostics, can not only be used for accurate diagnosis of tumors but also ablate tumors.

Based on these characteristics, Cai et al.12 developed pH-sensitive theranostic agents for precision detection and efficient therapy of tumors (Figure 11a). IR-PY was composed of a NIR heptamethine cyanine dye IR-822 that possessed high extinction coefficients and native preferential tumor accumulation property, and N1-(pyridin-4-ylmethyl)ethane-1,2-diamine (PY) as a pH-sensing receptor. In an acidic tumor microenvironment, the NIR fluorescence emission intensity (at 765 nm) of IR-PY enhanced due to the inhibition of the photoinduced electron-transfer process, which allowed the probe IR-PY with high spatial resolution for PAI in tumor and effective tumor photothermal ablation in vivo. Upon NIR 808 nm laser irradiation, the tumor in mice was remarkable ablated through PTT (Figure 11b). The “all in one” multifunctional agent has an extensive clinical prospect in NIRF/PA dual-modal imaging-guided cancer diagnosis and treatment.

Recently, an activatable theranostic probe for imaging-guided therapy has been developed by our group.9 The probe CyGal-P was designed by attaching β-galactose moiety on a NIR chromophore CyOH, which was linked by a long PEG chain as a hydrophilic group to promote in vivo biodistribution (Figure 11c). CyGal-P was initially non-fluorescence and non-photoacoustic signals. In the presence of β-galactosidase (β-Gal) that overexpressed in primary ovarian cancer cells, NIRF, PA and photothermal properties of CyGal-P were switched on, which was confirmed by NIRF and PA imaging in the tumors. In contrast, the control probe CyGal without conjugation of the PEG chain did not exhibit observable NIRF, PA and photothermal signals in the tumors (Figure 11d). Moreover, CyGal-P could effectively suppress cancer growth upon laser irradiation (Figure 11e) and no obvious histopathological abnormalities were found in normal tissues. This study thus offers a new approach towards activatable multimodal imaging-guided PTT.

Figure 11. (a) Schematic illustration of IR-PY as a versatile theranostic probe for pH sensing, tumor targeting, and NIRF/PA dual-modal imaging-guided PTT therapy. (b) The tumor growth curve for 30 days post-treatment for the different groups. Adapted from Ref. [12]. Copyright ©2017 Royal Society of Chemistry. (c) Schematic illustration of the activation mechanism of the macrotheranostic probe CyGal-P in βGal-overexpressing cancer cells. (d) Infrared thermal images of SKOV3 tumor-bearing mice under laser irradiation for different time after intravenous injection of CyGal-P and CyGal. (e) Tumor growth curves of mice after intravenous injection of saline, CyGal, and CyGal-P into SKOV3 tumor-bearing mice with or without laser irradiation at 680 nm. Adapted from Ref. [9]. Copyright © 2018 Wiley - VCH Verlag GmbH & Co. KGaA, Weinheim.

4. Conclusion

Theranostics has advantages in improving the treatment effect of cancer and reducing side effects. Hence, the development of effective theranostic agents is of vital importance in cancer therapy and evaluation of therapeutic efficacy. In this perspective, we have focused on the development of AMAs. The main design concept of current AMAs is to integrate imaging agents, analyte-specific cleavable linkers, therapeutic agents, and targeting groups. The “all-in-one” approach that combines different functional components in one platform has obvious advantages over that a single modality of
diagnosis or treatment because it can reduce side effects and minimize the probability of over-medication in tumor therapy. However, it also faces some problems that need to be solved: (i) Small molecules often encounter photobleaching issues under prolonged exposure, making AMAs less favorable for long-term clinical applications. In addition, the structures of AMAs are more complicated than normal photosensitizers. Hence, design of photostable imaging reagents with simple synthetic procedures is needed. (ii) How to improve the specific uptake of theranostic agents in tumor tissues is another question. Due to the high permeability of AMAs, AMAs have poor enrichment capacity in the tumor sites. (iii) The therapeutic performance of AMAs in tumor tissues is another question. Due to the high permeability of AMAs, AMAs have poor enrichment capacity in the tumor area, leading to low therapeutic effect. Hence, it is necessary to optimize the targeting ability of AMAs and use various specific targeting molecules or proteins to enhance the targeting effect of AMAs on tumor sites. (iii) The therapeutic performance of AMAs needs to be improved. Previous researches have shown that single therapy often has a limited therapeutic effect on tumors, while synergistic therapy can achieve the curative effect of “1+1>2”, which can overcome the tumor tolerance to single therapy. As for imaging, in the primary tumor and metastases for early diagnosis and therapy applications, various imaging modes have their advantages and application scope. Multi-mode imaging (such as FLI, PAI, and CLI) can overcome the false positives that may be caused by single-mode imaging and improve the accuracy of imaging and detection. Therefore, multi-mode imaging can achieve complementary advantages and better imaging results.

Nowadays, with the development of material technology, nanomedicine, and bioengineering, the integration of diagnosis and treatment of cancer will provide a new opportunity for mankind to conquer cancer.

Conflicts of interest
There are no conflicts to declare.

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