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Nanozymes for Catalytic Cancer Immunotherapy

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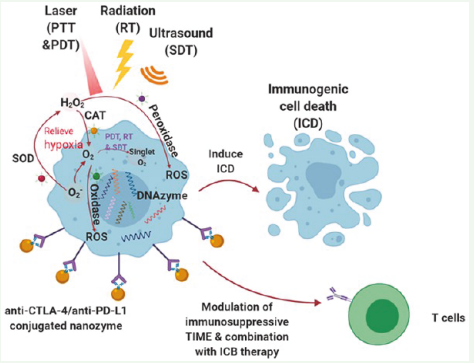
ABSTRACT: Nanozymes are nanoparticles with enzyme-mimicking properties. With the intrinsic catalytic properties, nanozymes are endowed with merits to modulate the immunosuppression of tumor immune microenvironment (TIME), mainly through altering reactive oxygen and nitrogen species (ROS/RNS) level. Besides redox imbalance, nanozymes can modulate other features of TIME, such as hypoxia. Modulations of TIME enabled by nanozymes can improve the effectiveness and efficiency of cancer therapies, especially immunotherapy. Two types of nanozymes, metal/metal oxide nanozymes and deoxyribozymes (DNAzymes) are currently investigated in cancer immunotherapy. They either act as standalone therapeutics or synergize with other therapeutic strategies to enhance antitumor effects. This Review summarizes the common nanozymes and their enzymatic properties, their interactions with TIME, and recent achievements of using nanozymes in cancer immunotherapy.



KEYWORDS: *nanozyme, cancer immunotherapy, immune checkpoint blockade therapy, tumor immune microenvironment, reactive oxygen species, hypoxia, DNAzymes*

1. INTRODUCTION

1.1. Cancer Immunotherapy and Tumor Immune Microenvironment. As tumors form and rapidly grow, the expression of tumor-associated antigens (TAAs) can be recognized by immune system.[1] Some tumors may be eliminated and undergo "Cancer Immunity Cycle": TAAs are released and captured by dendritic cells (DCs); DCs present captured TAAs to T cells, leading to the priming and activation of effector T cells; activated effector T cells infiltrate into tumor bed and recognize tumor cells; and targeted tumor cells are killed and release TAAs.[2] While other tumors can reach an equilibrium with the immune system in which immune system contains the growth of tumor. This equilibrium can be disturbed by immunosuppression and then the tumor can evade from immune responses.[3] On the basis of the cancer immunity cycle and immunosuppression, various immunotherapeutic strategies have been developed, such as vaccine, chimeric antigen receptor-T cell (CAR-T) therapy, and immune checkpoint blockade (ICB) therapy. Vaccines are designed to elicit immune responses or enhance immune responses by delivery of TAAs, immunostimulatory agents, antigen-presenting cells (APCs), or tumor cells.[4] Another encouraging strategy is to genetically engineering T cells with chimeric antigen receptor, which contains a binding domain for tumor cells and a activation domain for T cells. Upon binding to tumor cells, the engineered T cells can be activated and then proliferate to kill tumor cells. In addition to these strategies, recently developed ICB therapy attracted much attention. Unlike the above strategies that stimulate or directly use T cells, ICB therapy reverses immunosuppression and release the immune potentials of T cells. To prevent autoimmune responses, T cells and APCs express costimulatory receptor−ligand pairs to precisely regulate immune responses. And stimulation of T-cell is unlikely to occur without the presence of costimulatory receptor−ligand pairs.[5] However, cancer cells and other cells in the tumor immune microenvironment (TIME), such as myeloid-derived suppressor cells, express costimulatory or inhibitory ligands, and utilize this costimulation mechanism for their advantages to silence T cell activation.

The clinically advanced ICB therapy has been approved for cancer treatment by FDA since 2011 with the hope of eradication of cancers and durable responses to prevent recurrence.[6] However, ICB therapy is only effective for certain types of cancer, such as melanoma and nonsmall cell lung cancer and less than 13% US cancer patients respond to this therapy.[7] The resistance to immunotherapy can be partly ascribed to the heterogenicity and immunosuppression of TIME,[8] which is comprised of various cells, soluble factors, small molecules, and extracellular matrix (ECM) (Figure 1).[9,10] Both the cellular and molecular components of TIME

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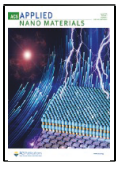

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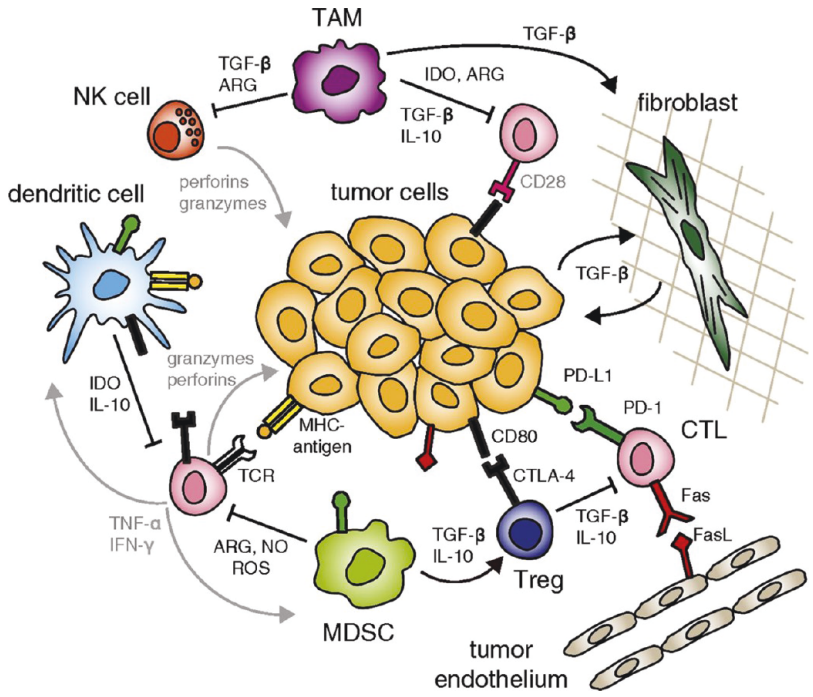

Figure 1. Immunosuppressive tumor immune microenvironment (TIME). NK cell: Natural killer cell. TGF-β: Transforming growth factor β. ARG: Arginase. TAM: Tumor-associated macrophage. IDO: Indoleamine 2,3-dioxygenase. CTL: Cytotoxic T lymphocyte. Treg: regulatory T lymphocyte. MDSC: Myeloid-derived suppressor cell. TCR: T cell receptor. Reprinted from ref 10 with permission from National Academy of Sciences. Copyright 2015.

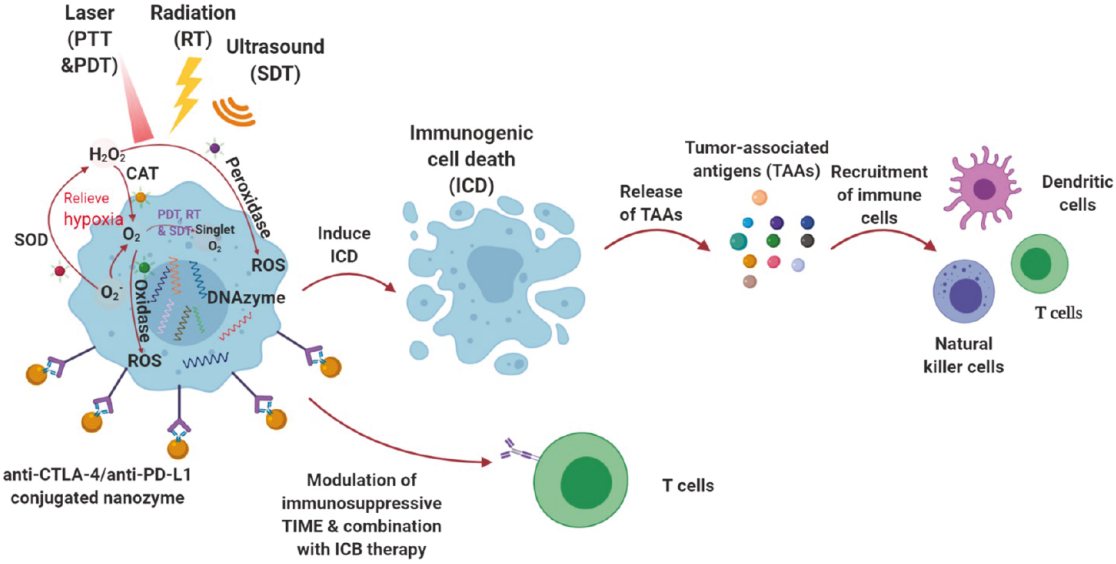

Figure 2. Nanozymes enhance cancer immunotherapy by two possible mechanisms: (1) induction of immunogenic cell death via standalone catalytic therapy or synergized with photothermal therapy (PTT), photodynamic therapy (PDT), sonodynamic therapy (SDT), and radiotherapy (RT), etc. and (2) modulation of the immunosuppressive TIME and reactivate immune responses.

can suppress immune responses, thus hindering the efficacy of immunotherapy.[11] Cancer cells or other cells in TIME expresses inhibitory ligands or secrete immunosuppressive molecules to prevent immune cells from recognizing or killing tumor cells. In addition, other features of TIME, such as hypoxia and acidity, have been reported to facilitate tumor evasion.[12,13] Therefore, targeting the cellular and molecular components of TIME is critical for cancer immunotherapy.

1.2. Enzyme Therapeutics and Nanozymes. TIME act as therapeutic barrier to immunotherapy because of its immunosuppressive nature. Modulation of TIME reverses immunosuppressive and thus enhance immunotherapy. As modulators of TIME, enzymes have been used as adjuvant therapeutics with the ability to degrade immunosuppressive molecules in the TIME or cleave immunosuppressive ligands on tumor cells, sensitizing cancer cells to immunotherapy.[14] For example, glycocalyx on tumor surface can be exploited by tumor cells to evade immune destruction. Sialidase that can cleave glycocalyx inhibits the binding of natural killer (NK) cells to tumor cells, rendering tumor cell killing by NK cells.[15]

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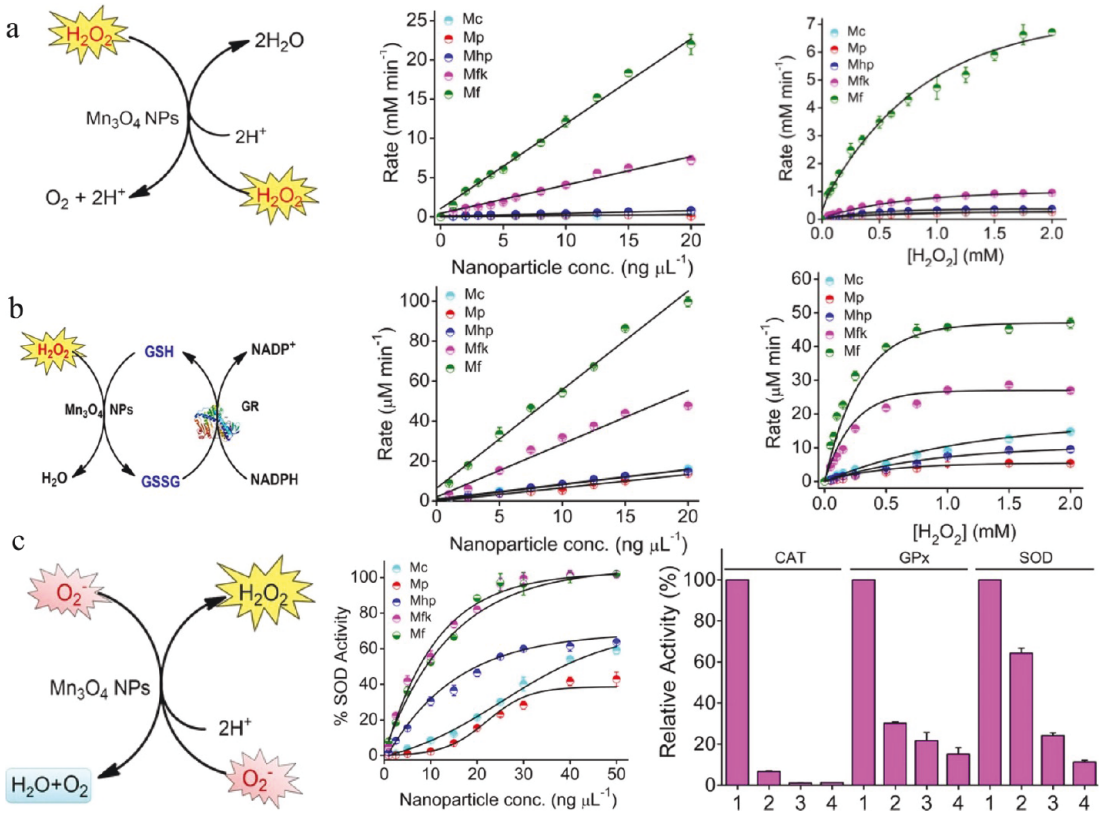

Figure 3. CAT-, GPx-, and SOD-like properties of Mn_3O_4 nanoparticles. (a) From left to right, the reaction mechanisms for CAT, effects of nanoparticle concentration and H_2O_2 concentration on the CAT-activity of Mn_3O_4 nanozyme, and the steady-state kinetic parameters for CAT-activity. (b) From left to right, the reaction mechanisms for GPx, effects of nanoparticle concentration and H_2O_2 concentration on the GPx-activity of Mn_3O_4 nanozyme, and the steady-state kinetic parameters for GPx activity. (c) From left to right, the reaction mechanisms for SOD, the effects of nanoparticle concentration on SOD activity of Mn_3O_4 nanozyme, and the comparisons of different manganese oxides for their enzymatic activity. 1: Mn_3O_4. 2: MnO_2. 3: MnO. 4: Mn_2O_3. Reprinted from ref 32 with permission from John Wiley and Sons. Copyright 2018.

In addition, from a metabolic perspective, cancer progression is caused by the redox imbalance because of 7the Warburg effect.[16] Enzymes which can alter the level of ROS can enhance the immunity against tumor. For example, catalase can protect T cells against oxidative stress and thus enhance the efficacy of cancer immunotherapy.[17]

Although effective, natural enzymes suffer from low stability, high cost, and difficult storage.[18] Recently, one emerging trend in cancer therapy is the utilization of nanomaterials as therapeutics because of their smaller size, better tumor penetration, and greater tumor accumulation compared to conventional therapeutic agents.[19] As one of these nanosized therapeutics, nanoparticles with enzyme-mimicking properties are superior to natural enzymes without the aforementioned limitations.[20] The concept of nanozymes can be dated back to 2004 in which Manea et al. reported ligand-functionalized gold nanoparticles loading with Zn^{2+} for phosphoester cleavage. Afterward, the discovery of Fe_3O_4 magnetic nanoparticles as a peroxidase mimic brings nanoparticle-based catalysts to a new era.[21] Since then, multiple nanozymes that can interfere with molecular components of TIME, such as reactive oxygen species (ROS), and cellular components of TIME, such as cancer cells, have been developed.

For catalytic cancer immunotherapy, nanozymes might be involved in the following two processes: induction of immunogenic cell death (ICD) by catalytic reactions to elicit immunity or modulation of immunosuppressive TIME to reactive immune responses (Figure 2). To summarize recent advances in catalytic cancer immunotherapy, first, we will exemplify representative nanozymes and discuss their enzymatic properties. Then the interactions of nanozymes with TIME will be discussed. Finally, the applications of nanozymes in cancer immunotherapy will be surveyed. Since nanoparticles only serve as delivery vehicle in natural enzyme-nanoparticle conjugation system, they are not considered as nanozymes in this Review.

2. COMMON NANOZYMES

Since 2007, various types of nanozymes, including metal-based, metal oxide-based, and carbon-based nanozymes, have been developed. Currently, only few natural enzymes can be mimicked by nanoparticles, including catalase (CAT), superoxide dismutase (SOD), peroxidase, oxidase, and phosphatase. In this section, based on their functions, common nanozymes are categorized into different types: antioxidant nanozymes, pro-oxidant nanozymes, and phosphoester-cleaving nanozymes. Antioxidants enzymes are essential in defense against oxidative stress and maintenance of cellular functions. In human body, endogenous enzymes, such as CAT, SOD, and glutathione peroxidase (GPx), are responsible for scavenging reactive species.[22] In contrast, pro-oxidant enzymes are enzymes that can induce oxidative and nitrosative stress and expose damage to lipids, membranes, DNA, and proteins.[23] Besides antioxidant and pro-oxidant enzyme mimics, some nanoparticles exhibit phosphatase-like properties and cleave phosphoester bond.[24] Unlike earlier reports on nanozymes that

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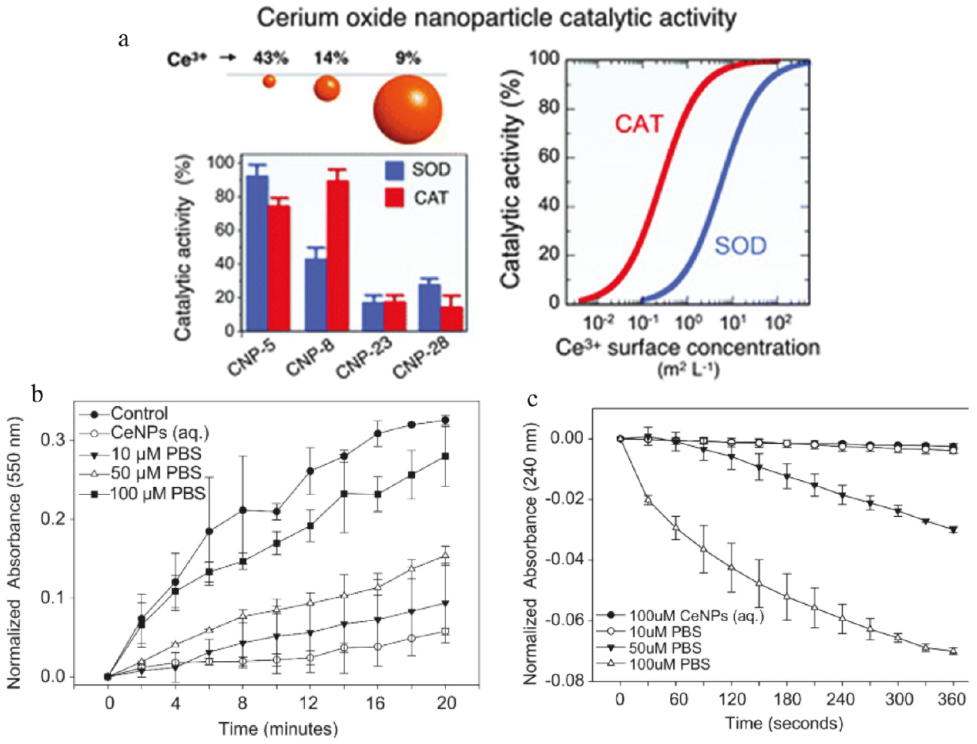

Figure 4. Effects of size, surface redox state, and local anion environment on the enzymatic property of nanoceria. (a) Within a size range, the amount of Ce^{3+} on the surface of nanoceria decreases with size increasing. Nanoceria with higher Ce^{3+} tend to exhibit SOD-like property while CAT-like property is obtained with lower Ce^{3+}. Reprinted from ref 33 with permission from Royal Society of Chemistry. Copyright 2018. (b) SOD activity of nanoceria is reduced after addition of phosphate ions. (c) CAT activity of nanoceria is enhanced in the presence of phosphate ions. Reprinted from ref 35 with permission from Elsevier. Copyright 2011.

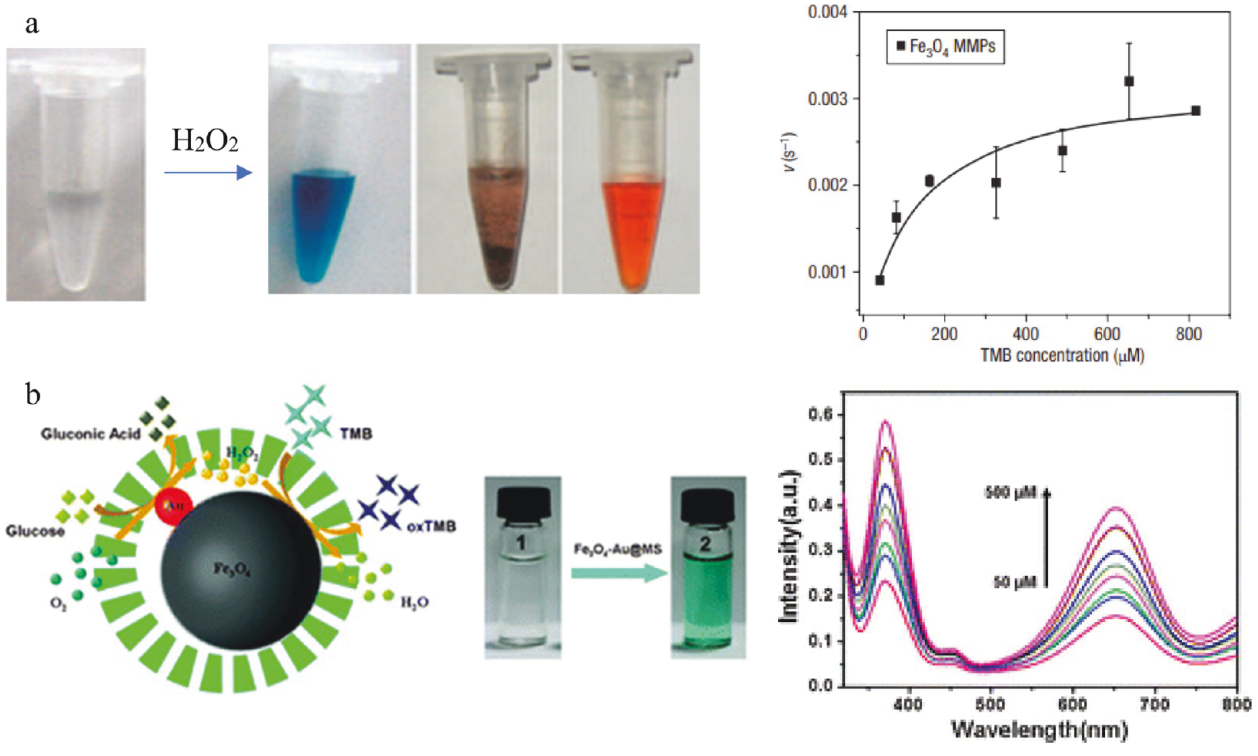

Figure 5. Pro-oxidant properties of Fe_3O_4 and Au−Fe_3O_4 nanozymes. (a) Fe_3O_4 nanoparticles possess peroxidase-like activity and can catalyze the oxidation of common HRP substrates into colorful products. And the catalysis of TMB oxidation follows a typical Michaelis−Menten kinetics. Reprinted from ref 21 with permission from Springer Nature. Copyright 2007. (b) Au−Fe_3O_4 nanoparticles mimic the glucose oxidase and peroxidase enzymatic cascade. Reprinted from ref 36 with permission from Royal Society of Chemistry. Copyright 2013.

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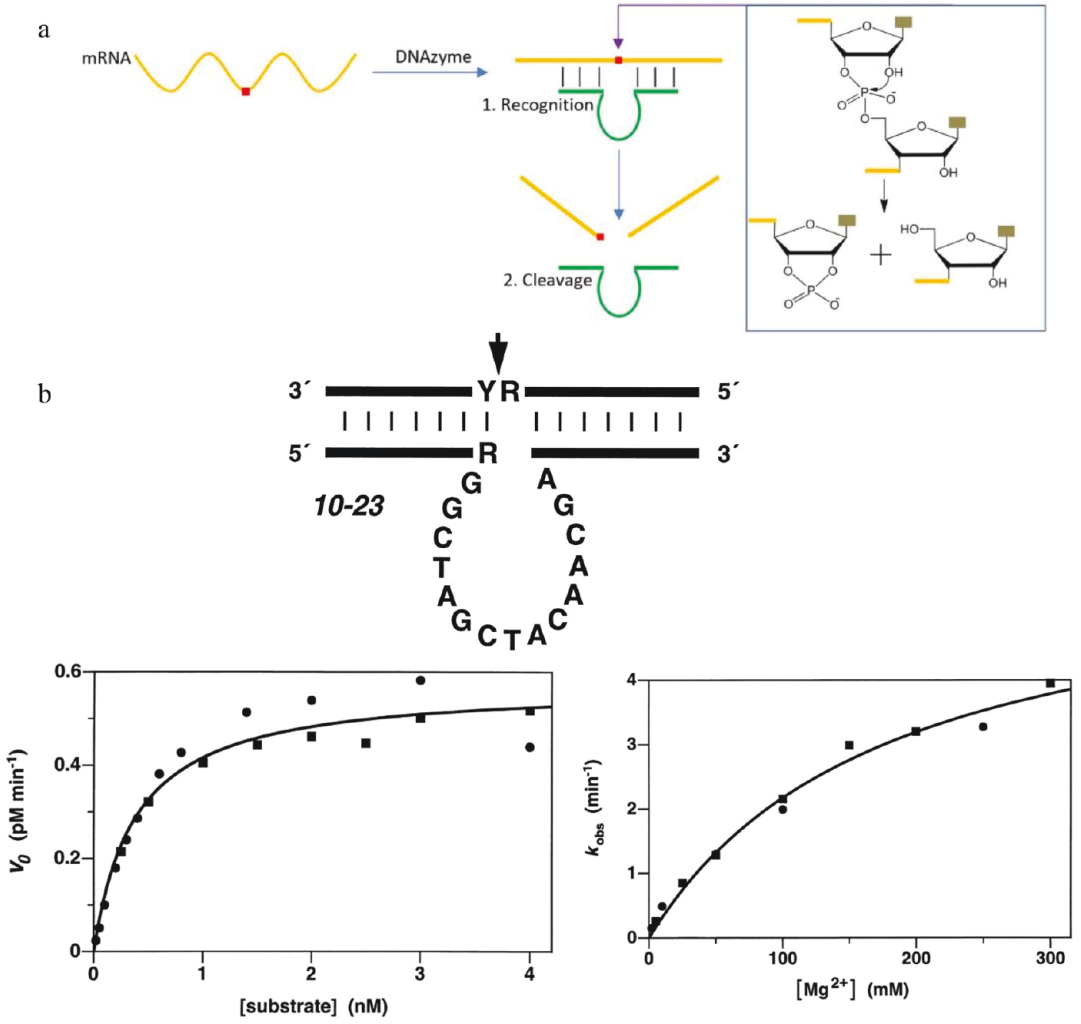

Figure 6. General working principle of DNAzymes and the kinetic study of a 10−23 DNAzyme. (a) Principle of mRNA recognition and cleavage by DNAzymes. Reprinted from ref 31 with permission from Ivyspring. Copyright 2017. (b) Structure and kinetic analysis of a 10−23 DNAzyme. The arrow indicates the cleavage site. Y = U or C. Reprinted from ref40 with permission from National Academy of Sciences, U.S.A. Copyright 1997.

mainly focus on metals and metal oxides, here, we extend the concept of nanozymes into a broader range and discuss RNA-cleaving DNAzymes. Recently, a new type nanozyme-single atom-nanozymes with well-defined active centers has become the frontiers in the nanozyme research. Therefore, in this section, single-atom nanozymes will also be discussed. Numerous nanoparticles for mimicking the above enzymes have been summarized in other reviews,[25−31] and here, we only discuss few representative nanozymes and their properties for each category.

2.1. Antioxidant Nanozymes. SOD and CAT are responsible for scavenging ROS and serve as antioxidants. SOD catalyzes superoxide (O_2^-) to produce H_2O_2 and O_2 and CAT further decompose H_2O_2 into harmless H_2O and O_2. Unlike SOD and CAT, GPx converts H_2O_2 into water in the presence of reduced glutathione (GSH) without producing O_2. One exemplary nanoparticle that can mimic multiple antioxidant enzymes is manganese oxide. In one report, Mn_3O_4 nanoparticles could mimic SOD, CAT, and GPx and their catalytic activity depends on various factors including size, morphology, surface area, and redox properties of the surface metal ions.[32] In Figure 3, Mn_3O_4 with different morphologies were synthesized and their steady-state enzyme kinetics were also studied. Mn_3O_4 nanoparticles followed a typical Michaelis−Menten reaction kinetics for CAT (Figure 3a) and GPx (Figure 3b). Nanoflowers (Mf) showed higher ROS-scavenging activity over other morphologies and Mn_3O_4 (1) had higher activity than other oxides of Mn, such as MnO_2 (2), MnO (3), and Mn_2O_3(4) (Figure 3c).

Another prominent example of antioxidant nanozyme is nanoceria. Nanoceria can also mimic both SOD and CAT and their enzymatic activity highly depends on their surface redox state, size, and local anion environment. Nanoceria with higher Ce^{3+}/Ce^{4+} ratio on the surface exhibits SOD-like activity. In contrast, with a lower Ce^{3+}/Ce^{4+} ratio, nanoceria exhibits CAT-like property. The redox state of nanoceria surface also correlates with the size and within a size range, nanoceria with a larger size have a lower Ce^{3+}/Ce^{4+} ratio (Figure 4a),[33] which is consistent with another study.[34] In addition to surface redox state and size, another important factor affecting the enzymatic property of nanoceria is the local anions. It has been shown that phosphate ion (PO_4^{3-}) can decrease SOD activity (Figure 4b) and increase CAT activity (Figure 4c) of nanoceria which are even with predominant Ce^{3+} redox state. It was proposed that the formation of cerium phosphate on the surface of

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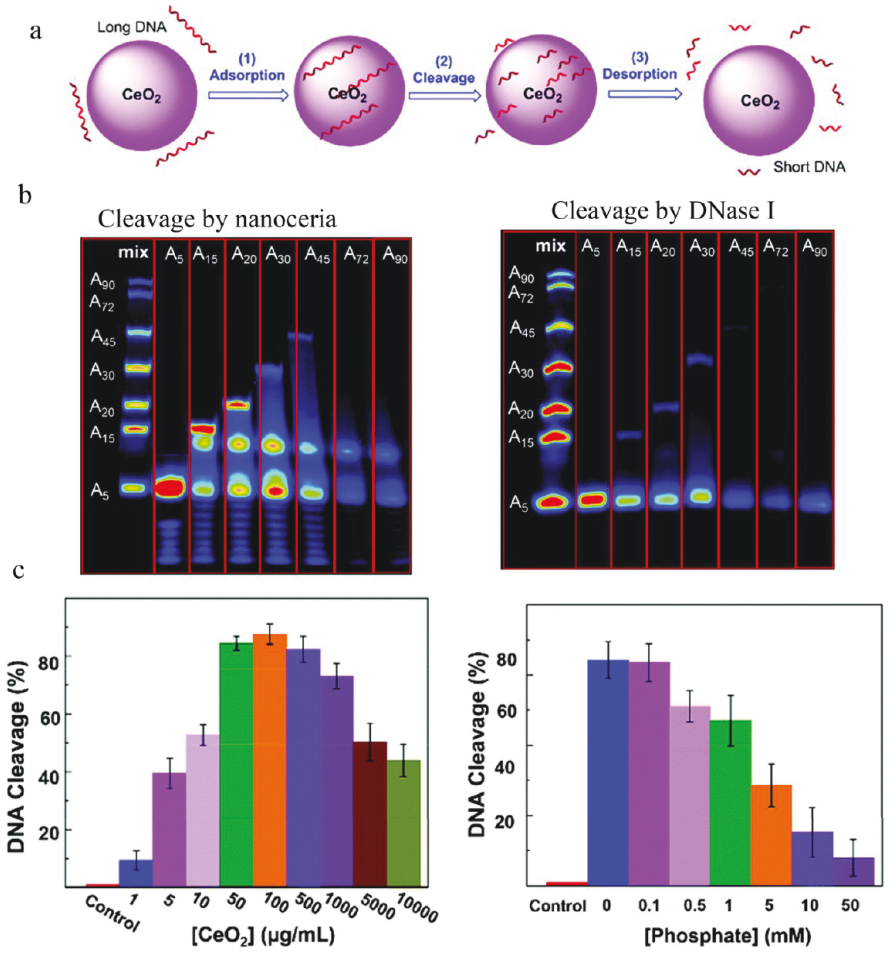

Figure 7. Phosphatase-like activity of nanoceria. (a) Schematic of DNA cleavage by nanoceria including DNA adsorption, cleavage, and desorption. (b) Similar cleavage pattern of nanoceria and DNase I. (c) Effects of nanoceria concentration and phosphate concentration on DNA cleavage yield of nanoceria. Reprinted from ref 41 with permission from Royal Society of Chemistry. Copyright 2019.

nanoceria trapped the ceria in +3 state and blocked the cycling of +3 and +4 redox state, leading to decreased SOD activity.[35]

2.2. Pro-oxidant Nanozymes. Peroxidase and oxidase are pro-oxidant enzymes that can induce oxidative and nitrosative stress. In 2007, Yan group discovered the Fe_3O_4 nanoparticles as peroxidase mimic for the first time.[21] In this study, with the presence of H_2O_2, Fe_3O_4 nanoparticles catalyzed the oxidation of common horseradish peroxidase (HRP) substrates including 3,3′,5,5′-tetramethybenzidine (TMB), *o*-phenylenediamine dihydrochloride (OPD), and 3,3′-diaminobenzidine tetrahydrochloride (DAB) into colorful products and the TMB oxidation reaction followed a typical Michaelis−Menten kinetics (Figure 5a). The Fe_3O_4 nanozyme could catalyze the oxidation of TMB with a higher binding affinity than HRP, demonstrating the robustness of Fe_3O_4 as peroxidase mimic. To combine the Fe_3O_4 nanozyme with other pro-oxidant nanozymes, He et al. reported a core/shell composite nanozyme comprised of Au−Fe_3O_4 nanoparticle complex as core and mesoporous SiO_2 as shell (Figure 5b).[36] Fe_3O_4 nanoparticles served as peroxidase mimic and Au nanoparticles acted as oxidase mimic. The outer SiO_2 shell layer could hinder the aggregation and make this nanozyme system stable even in harsh conditions. In the presence of glucose and O_2, Au nanoparticles catalyzed the oxidation of glucose into gluconic acid and H_2O_2 and then Fe_3O_4 nanozyme catalyzed the oxidation of TMB into colorful products with the presence of H_2O_2. This nanoparticle system simulated the enzymatic cascade for glucose oxidase and peroxidase for the first time in one system.

2.3. Phosphoester-Cleaving Nanozymes. With the development of nucleic acid technology, nanosized oligonucleotides have been widely adopted as therapeutics. As one example, DNAzymes with the ability to cleave RNA are explored as diagnostic and therapeutic tools for various applications, such as biosensing and gene silencing.[37] Although some ribozymes can also cleave RNA, higher susceptibility to cellular ribonucleases, higher cost, and toxicity compared to DNAzymes, hamper their applications for in vivo RNA cleavage.[38] Therefore, in this Review, we focus on DNAzymes for RNA cleavage. By designing substrate binding arms using Watson−Crick base pairing, the DNAzyme can recognize specific RNA and 2′-hydroxyl group of RNA attacks the adjacent phosphodiester bond to initiate the cleavage (Figure 6a).[31] During this process, metal ions are involved and assist the deprotonation of 2′-hydroxyl in the target RNA.[39] Therefore, metal ions are usually necessary as cofactors for DNAzymes.

In an early study, Joyce and co-workers synthesized a10−23 DNAzyme that could be tailored to cleave almost any RNA substrate under physiological conditions.[40] This DNAzyme was comprised of a catalytic core and two substrate-recognition domains on left and right flanks. Through base pairing, target

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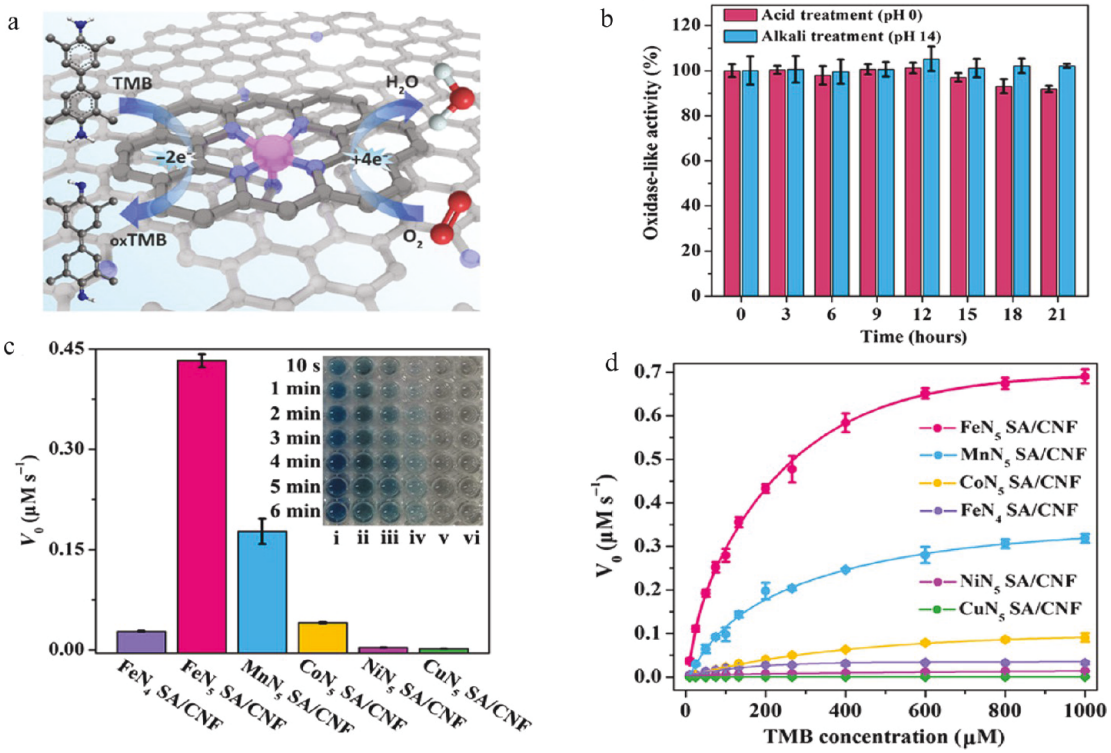

Figure 8. (a) Scheme for FeN_5 SA/CNF-catalyzed TMB oxidization. (b) Robust enzymatic performance of FeN_5 SA/CNF in extremely low and high pH. (c) Comparisons of initial reaction rates of FeN_5 SA/CNF nanozymes with other metal-based SANs. (d) MN_5 SA/CNF nanozymes catalyzed TMB oxidization reaction followed typical Michaelis−Menten kinetics. Here, M is Mn, Fe, Co, Ni, and Cu. Reprinted from ref 45 with permission from American Association for the Advancement of Science. Copyright 2019.

RNA was cleaved at a particular phosphodiester located between an unpaired purine and a paired pyrimidine residue (Figure 6b). The catalytic activity was dependent on substrate and cofactor concentration. At fixed DNAzyme concentration (0.004 nM) and varied concentrations of substrate (0.02−4 nM), the plot of initial reaction rate versus substrate concentration followed a typical Michaelis−Menten kinetics. As the cofactor, Mg^{2+} also affected the catalytic rates of this DNAzyme in a concentration-dependent manner (Figure 6b).

In addition to DNAzymes, some nanoparticles possess phophoester-cleaving activity. Nanoceria is one of the most explored phosphatase-mimicking nanozymes. Cerium ion (IV) and nanoceria have long been known to act as phosphatase to cleave phosphoester. Xu et al. discovered that nanoceria with a size of ∼5 nm could mimic DNase I, which cleaves DNA at phosphodiester linkage adjacent to a pyrimidine nucleotide.[41] CeO_2 can strongly adsorb DNA through the binding of DNA phosphate backbone to cerium ions on the surface (Figure 7a). The cleaved products of different DNA fragments with various length are very similar to those of DNase I, suggesting the DNase I-like activity of nanoceria (Figure 7b). Since it is already known that Ce^{4+} ions can cleave DNA, to test if nanoceria exhibits the DNA-cleavage activity, the authors centrifuged nanoceria solution and redispersed the pellet. The nanoceria pellet showed DNA cleavage ability, which demonstrates that nanoceria is responsible for the cleavage of DNA in this study. The DNA cleavage yield is in a concentration-dependent manner (Figure 7c left). In addition, the authors showed that phosphate ions (PO_4^{3-}) could inhibit the DNase activity of nanoceria (Figure 7c right) since $PO4^{3-}$ ions can strongly absorb on the surface of nanoceria and compete with DNA for the surface.

2.4. Single-Atom Nanozymes. Although nanozymes are advantageous over natural enzymes, drawbacks such as low selectivity and catalytic ability, still exist. To resolve these issues, a new type of nanozyme, single-atom nanozymes (SANs) have been introduced.[42] SANs are featured with metal atomically dispersed on solid support and the metal−support interactions promote a low-coordination environment and charge-transfer effects, leading to a higher catalytic activity of the active sites.[43] Iron atoms dispersed on nitrogen-doped carbon nanomaterial are one of the widely used SANs.[44−46] Recently, a SAN with axial N-coordinated single-atom Fe (FeN_5) as active centers and carbon nanoframe as support (FeN_5 SA/CNF) were reported.[45] The active centers of this SAN resembled the heme-coordinated redox enzyme-cytochrome P450. Then oxidase model reaction was used as a proof-of-concept to explore the enzymatic property of the SAN (Figure 8a). The SAN was robust and stable even in harsh conditions such as extremely low pH and high pH (Figure 8b). Then the authors compared the Fe-based SAN with other metal-based SANs and found out that FeN_5 SA/CNF exhibited the highest oxidase activity. The reaction kinetics of these SANs with various concentrations of TMB, followed a typical Michaelis−Menten curve (Figure 8c and d).

3. NANOZYME-TUMOR IMMUNE MICROENVIRONMENT INTERACTIONS

TIME are featured with oxidative stress, hypoxia, acidity, glutathione (GSH), etc., and cancer cells utilize these features for their advantage to escape from immune destruction. Moreover, the TIME play a determinative roles in tumor survival and progression and therefore, normalization of TIME

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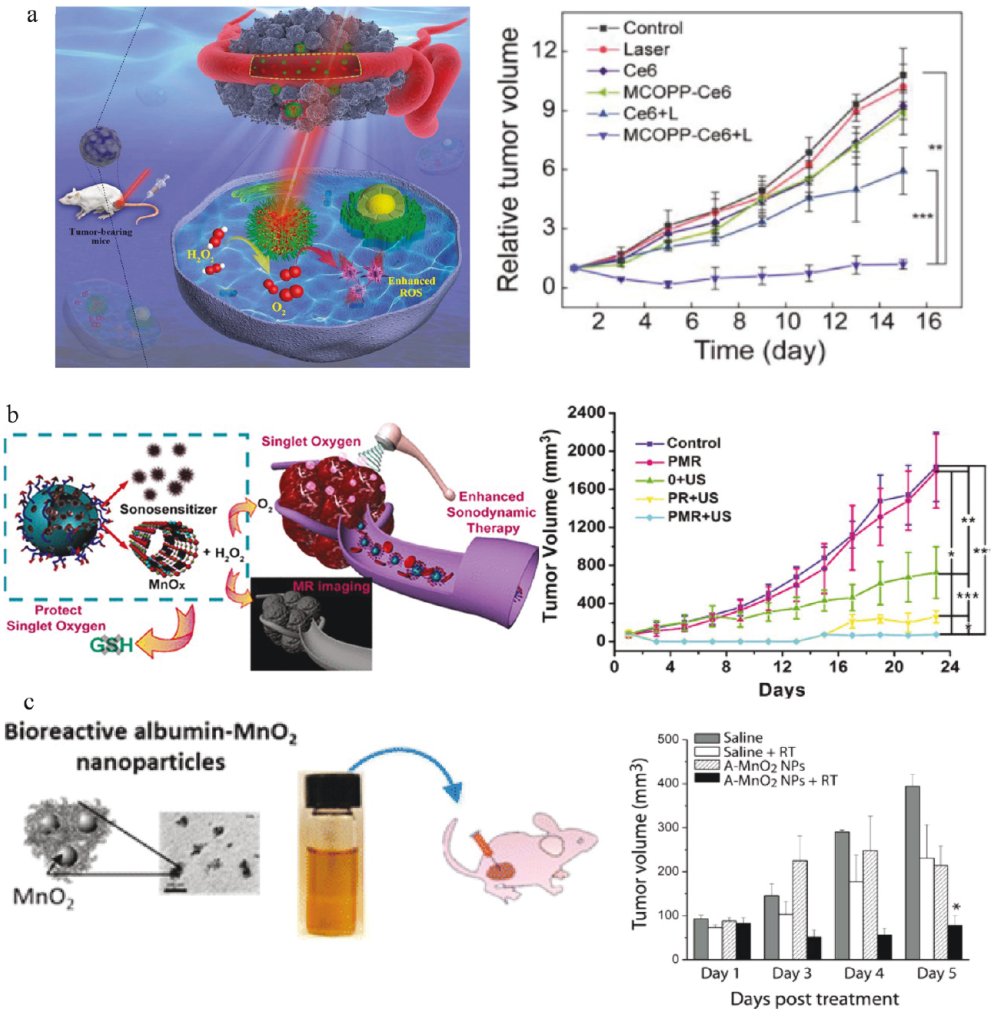

Figure 9. Mn-based nanozymes with catalase-like activity synergized with PDT, SDT, and RT for cancer therapy. (a) Schematic of MnCo-MOF nanozyme-enhanced PDT. Tumor volume was significantly reduced after the synergized treatment. Reprinted from ref 69 with permission from John Wiley and Sons. Copyright 2019. (b) Schematic of HMON-MnO*x* nanozyme-enhanced SDT. After injection of this nanozyme under the ultrasound irritation, no obvious tumor was observed at day 23. Reprinted from ref 70 with permission from American Chemistry Society. Copyright 2018. (c) Albumin−MnO_2 nanozyme enhanced RT. This nanozyme system could relieve hypoxia, acidosis, and downregulate HIFα and VEGF. The neutralization of acidity was achieved by an intermediated MnOOH with the ability to consume H^+. The oxygenation alone could significantly reduce tumor volume after injection of the nanozyme for 48 h. Upon X-ray radiation, the efficiency of RT was also significantly enhanced. Reprinted from ref 71 with permission from American Chemistry Society. Copyright 2014.

can improve cancer therapeutic efficiency.[47,48] Although other nanomaterials have been extensively used in regulating TIME, they usually serve as delivery vehicles for therapeutic agents.[49] In contrast, nanozymes can act as therapeutic enzymes as well as delivery vehicles for other agents. Because of their intrinsic enzymatic properties to modulate immunosuppressive molecules or features, such as ROS and hypoxia, nanozymes have been considered as promising therapeutics for normalizing TIME. In this section, the immunosuppression of different components in TIME will be discussed and then interactions of nanozymes with these components to modulate TIME for cancer treatment will be discussed.

3.1. ROS-Based Interactions. ROS and RNS are critical signaling molecules and regulate several physiological functions, such as vascular tone, insulin synthesis, cell profanation, differentiation, and migration.[50] However, in TIME, ROS and RNS are exploited by cancer cells to maintain cancer cell survival and render immunosuppression.[51] On the other hand, the overaccumulated ROS and RNS can also induce cancer cell death. Elevated level of ROS in cancer cells is resulted from the imbalance of production and elimination mainly due to the altered redox potential by aerobic metabolism. The accumulated superoxide (O_2^-) reacts with nitric oxide (NO) to produce another powerful oxidant-peroxynitrite ($ONOO^-$).[52] Beyond their traditional role to initiate cancer, ROS and RNS can also target immune cells in TIME to mediate cancer immunosuppression.[53−57] As the major immunosuppressive cells in TIME, tumor-associated macrophages (TAMs) and myeloid-derived suppressor cells (MDSCs) produce ROS and RNS via the metabolism of L-arginine by inducible nitric oxide synthase (iNOS) or arginase I to modulate immune cells, such as T-cells[57−64] and natural killer (NK) cells.[58,65] ROS can also up-regulate the expression of immune checkpoint PD-L1 on TAMs to exert their immunosuppressive effects.[54] In addition to its immunosuppressive roles, ROS are effector molecules for cancer cell killing in various therapeutic strategies, including chemotherapy, photodynamic therapy, sonodynamic therapy, and radio-

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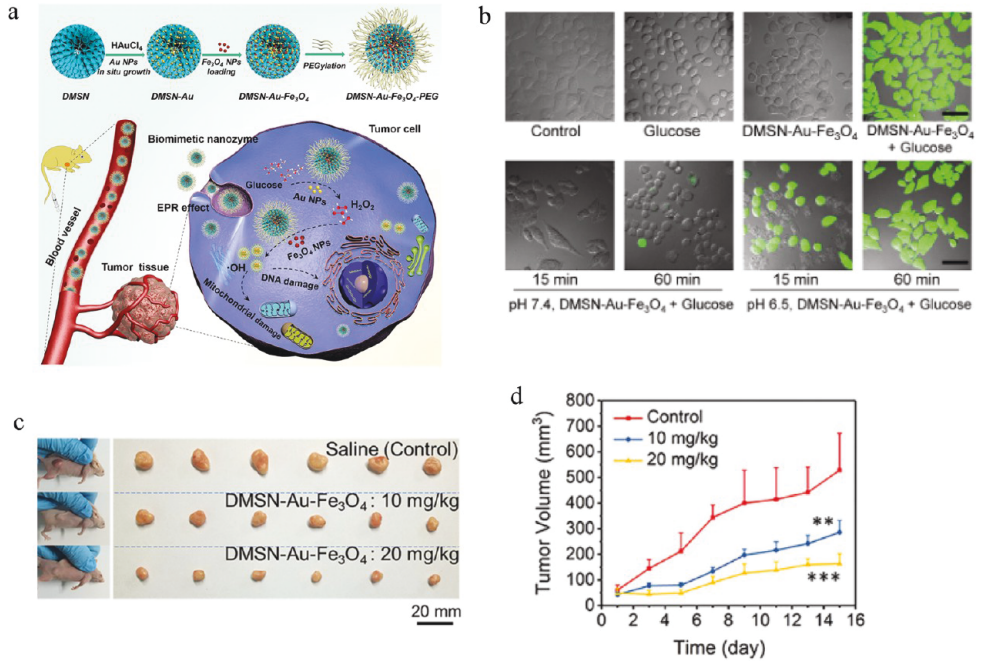

Figure 10. ROS-generating Au and Fe_3O_4-coloaded dendritic mesoporous silica nanoparticles for cancer therapy. (a) Schematic of nanoparticle synthesis and catalytic cancer therapy. (b) ROS assay for the nanozyme-treated cancer cells. Green fluorescence indicated the existence of ROS. (c) Change of tumor volume at the 15th day after injection of the nanozyme. (d) Time-dependent tumor volume curve. Reprinted from ref 83 with permission from John Wiley and Sons. Copyright 2019.

therapy. Therefore, targeting ROS/RNS is a widely employed strategy in cancer therapy.

Although RNS are also essential components of TIME, few RNS-scavenging nanozymes have been reported in cancer therapy despite their applications in traumatic brain injury[66,67] and osteoarthritis.[68] Due to the dual roles of ROS in TIME, two nanozyme-based therapeutic strategies are employed to treat cancers: scavenging ROS to relieve the oxidative stress of TIME or generating ROS to induce cancer cell death.

ROS-scavenging nanozymes usually exhibit CAT-like activity and produce O_2. Since O_2 is critical for photodynamic therapy (PDT),[69] sonodynamic therapy (SDT),[70] and radiation therapy (RT),[71] which utilize O_2 and sensitizers to generate ROS (mainly singlet O_2) upon the excitation of external energy, CAT mimics which can covert H_2O_2 to O_2, are synergized with PDT, SDT, and RT to enhance their therapeutic efficiency. Owning to that natural manganese catalase contains two manganese ions which act as catalytic active sites for H_2O_2 disproportionation,[72] manganese oxide nanoparticles have been the predominant catalase mimics.[9,69−71,73−79] Zhao and co-workers designed a mesoporous manganese cobalt oxide nanozyme derived from metal−organic frameworks (MOFs) that can mimic CAT property and decompose H_2O_2 to O_2 (Figure 9a).[69] This nanozyme could generate intracellular O_2 and downregulate the expression of hypoxia inducible factor HIF-1α in cancer cells, indicating the attenuation of the hypoxic condition. Then the authors tested the PDT treatment efficiency by using this nanozyme-conjugated photosensitizer Ce6 both in vitro and in vivo. Results showed that upon the excitation of laser, this nanozyme system significantly induce the death of cancer cells in vitro and reduced the tumor weight in a mice model.

Zhu et al. constructed a hollow mesoporous organosilica nanoparticles (HMONs) and MnO*x* nanoparticles were in situ generated in the mesopore channels of HMONs based on the strong oxidation potential of MnO_4^- and reduction potential of CTAC (cetyltrimethylammonium chloride) (Figure 9b).[70] The sonosensitizers PpIX were loaded onto HMONs-MnO*x* nanoplatform. O_2 production of this system was highly dependent on the Mn concentration and upon the ultrasound irradiation, singlet O_2 production was observed by the electron spin resonance (ESR) spectra. The live/dead assay showed that under ultrasound irradiation, this nanozyme system significantly induced cancer cell death compared to the controls. In a mice model, 23 days after the treatment, the tumor growth inhibition was as high as 96%.

Polyelectrolyte-albumin complex-coated MnO_2 nanoparticles were synthesized to enhance the therapeutic efficiency of radiotherapy due to the intrinsic CAT-like activity of MnO_2 nanoparticles (Figure 9c).[71] In addition to generate O_2 and relieve the hypoxic TIME, this nanozyme system could reduce acidity and downregulate HIF1α and vascular endothelial growth factor (VEGF). Albumin prevented the aggregation of MnO_2 nanoparticle in physiological fluids and rendered the cellular uptake of these nanoparticles. MnO_2 reacted with H_2O_2 to produce intermediate MnOOH which consumed H^+, thus neutralizing the acidic TIME. In a mice model, upon the radiation of X-ray, tumor volume reached ~78 mm^3 after 5 days, while control group reached ~231 mm^3.

In contrast to ROS-scavenging nanozymes, peroxidase and oxidase mimics have also been exploited to increase ROS level to destruct cancer cells.[20,80−84] Unlike ROS-scavenging nanozymes, which combines with PDT, SDT and RT to convert O_2 to toxic ROS, ROS-generating nanozymes themselves can usually serves as therapeutics. Fe_3O_4 nanoparticles doped with other metals showed significantly higher affinity to H_2O_2 than bare Fe_3O_4 nanozyme, with the ability to generate ROS even at a ultralow H_2O_2 level.[81] Other than nanozymes that exhibit the properties of one enzyme, dual-enzyme mimicking nanoparticles are attractive strategies for

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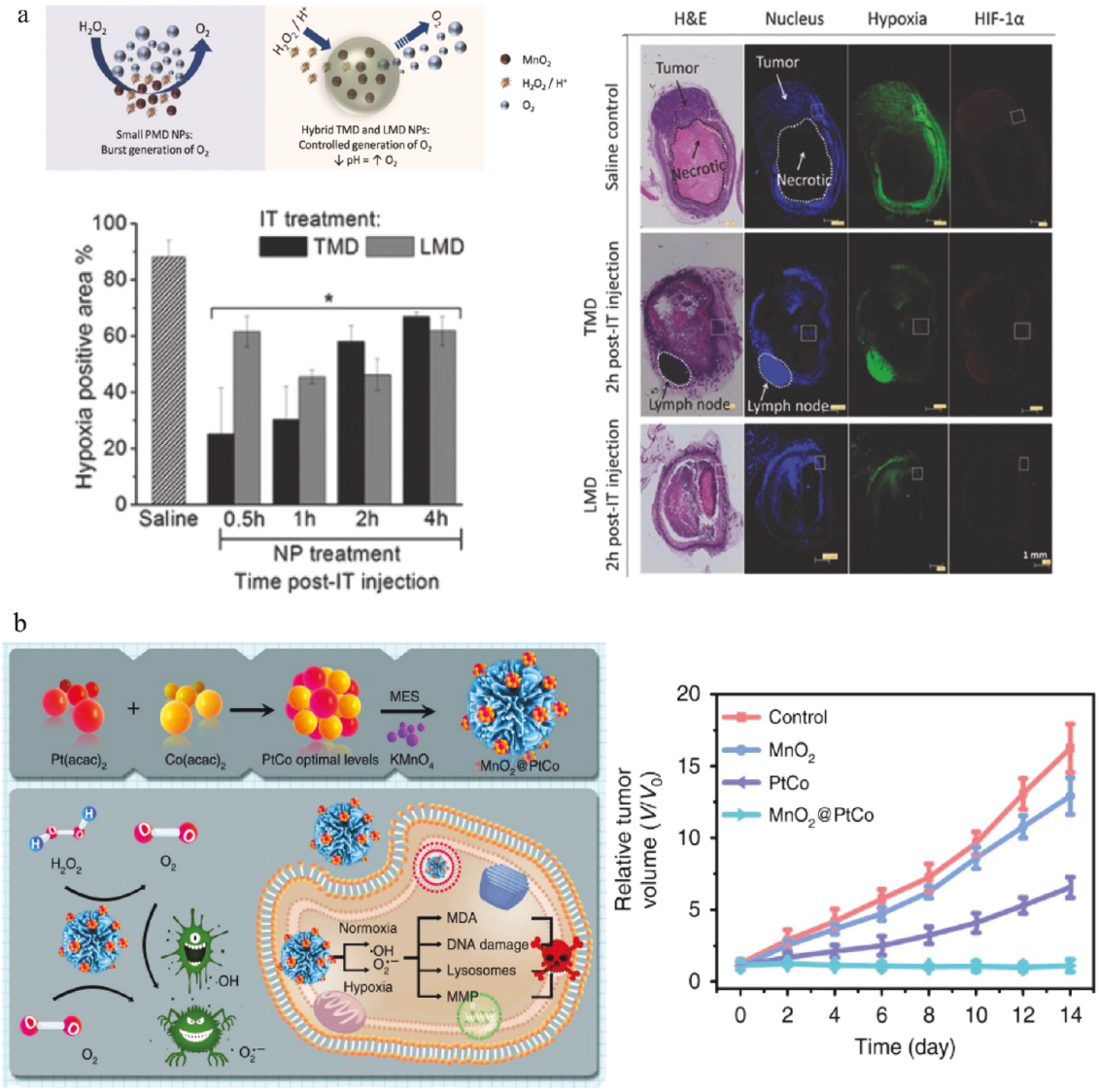

Figure 11. Hypoxia-alleviated nanozymes. (a) MnO_2-polymer nanozyme. From left to right, schematic of O_2 generation mechanism, H&E staining of hypoxia area, and quantification of hypoxia in tumor tissue. Reprinted from ref 92 with permission from John Wiley and Sons. Copyright 2015. (b) MnO_2@PtCo nanozyme. From left to right, the scheme of the synthesis process and working principle and the quantitation of tumor volume for different treatments. Reprinted from ref 93 with permission from Springer Nature. Copyright 2018.

catalytic cancer therapy. PtFe@Fe_3O_4 nanorods possess both peroxidase-like and catalase-like activities. As peroxidase mimic, the nanorods can decompose H_2O_2 to produce ROS to kill cancer cells, while as catalase mimic, the nanorods can decompose H_2O_2 into O_2 to reverse the hypoxic TIME. More importantly, the catalytic properties of this nanorods can be enhanced under the excitation of NIR laser. Combined the photothermal effects with catalytic effects, the tumor volume of a mouse deep pancreatic cancer was reduced by 99.8%.[80] Ultrasmall Au and Fe_3O_4 nanoparticles-coloaded dendritic mesoporous silica nanoparticles are able to mimic glucose oxidase and peroxidase (Figure 10).[83] The Au nanoparticles mimic glucose oxidase and catalyze glucose into gluconic acid and H_2O_2. The continuous generation of H_2O_2 provides substrate for peroxidase-like Fe_3O_4 to produce toxic ROS. This dual-enzyme mimicking nanoparticles have a desirable cancer suppression rate. The ROS assay showed that after treatment by this nanozyme for 60 min, cellular ROS was significantly increased. The mice tumor model also demonstrated the effectiveness of this nanozyme as therapeutics for cancer treatment at day 15 after the injection of the nanozyme. Another important example of ROS-generating nanozyme is the nitrogen-doped porous carbon nanosphere (N-PCNSs) which can exhibit four enzyme-like activities. When delivered into cell lysosome, this nanozyme only perform activities for ROS geneneration.[85]

3.2. Hypoxia-Based Interactions. As another important feature of TIME, hypoxia is a major driver for immunosuppression. Hypoxia can impair anticancer immunity by altering the functions of immune cells or by increasing cancer resistance to immune effectors.[86] For example, hypoxia can promote immunosuppression by inhibiting $CD4^+$ T cell function while enhancing Treg activity.[87] Hypoxia can also impair NK cell function by overexpressing CD73 which converts extracellular adenosine monophosphate (AMP) into immunosuppressive adenosine.[88] In addition, hypoxia is able to induce the expression of immune checkpoints and dampen the functions of T cells.[89] On the other hand, respiratory hyperoxia

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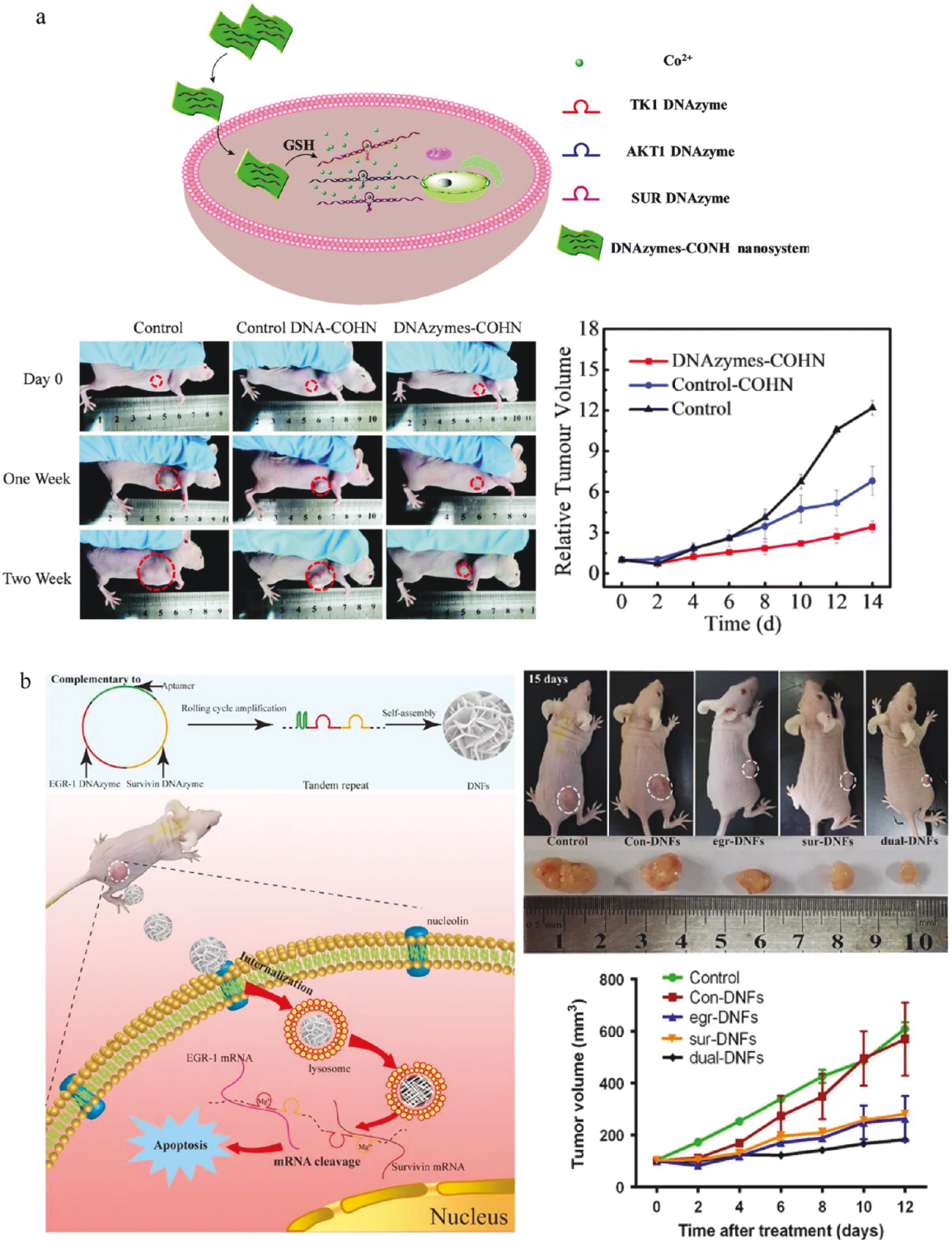

Figure 12. Nanostructure-supported DNAzymes for multiplexed gene silencing for cancer therapy. (a) GSH-responsive CoOOH nanoparticle conjugated with multiple DNAzymes for gene silencing. Cancer cell invasion and tumor volume was inhibited by the DNAzymes. Reprinted from ref 105 with permission from Royal Society of Chemistry. Copyright 2017. (b) DNAzme-incorporated DNF for enhanced cancer therapy. The tumor size was obviously shrunk by the DNFs. Reprinted from ref 104 with permission from Springer Nature. Copyright 2017.

mitigates adenosine-driven immunosuppression[90] and decreases the proportion of MDSCs and expression of PD-L1.[91] Therefore, oxygenation can effectively alleviate the immunosuppressive TIME. Besides its role in boosting immune responses, oxygen is also an important source for ROS, either through synergism with other therapies, such as PDT, SDT, and RT, or through oxidation reaction catalyzed by oxidase. In previous section, CAT-like nanozymes are usually synergized with PDT, SDT, and RT. In this section, we focus on CAT nanozymes serving as therapeutics without external energy sources.

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In a hybrid MnO_2-polymer nanoparticle system, two different hybrid nanoparticles were constructed by embedding polyelectrolyte-MnO_2 (PMD) in hydrophilic terpolymer/protein-MnO_2 (TMD) or hydrophobic polymer/lipid-MnO_2 (LMD) (Figure 11a).[92] The CAT-like activity enables MnO_2 nanoparticles to decompose H_2O_2 to generate O_2. Moreover, under acidity, TMD and LMD showed prolonged O_2 release. Both nanoparticles could be effectively uptaken by cells and no toxicity was observed both in vitro and in vivo. Compared to TMD, LMD showed better accumulation and retention in tumor tissue. Both TMD and LMD could significantly relieve hypoxia in a breast cancer tumor model.

Qu and co-workers designed a nanoflowers comprised of PtCo nanoparticles and MnO_2 nanoparticles (Figure 11b).[93] PtCo nanoparticles behave like oxidase, while MnO_2 nanoparticles function as CAT. The O_2 produced by outer layer MnO_2 nanozyme triggers the generation of ROS by inner PtCo layer. In this way, this nanozyme system can generate O_2 to alleviate hypoxia, as well as produce free radicals to induce cancer cell apoptosis. Under hypoxia, only MnO_2@PtCo nanozyme could induce obvious intracellular ROS generation, while PtCo alone produced less ROS due to the absence of O_2, which suggested that MnO_2 nanozyme could alleviate hypoxia. In the following in vivo study, this nanozyme significantly inhibited the tumor growth compared to other groups.

3.3. Phosphoester Cleavage-Based Interactions. Besides molecular components, various cells in TIME contribute to tumorigenesis and immunosuppression. Endowed with RNA-cleaving property, DNAzymes can alter cellular functions and thus modulate TIME. Of these DNAzymes, 10−23 DNAzyme is the most well characterized subtype and cleaves RNA at phosphoester bond.[94] The first report of using DNAzyme for cancer treatment was performed by targeting *bcr-abl* mRNA. Bcr-abl is a fused gene and found in most patients with chronic myelogenous leukemia. The DNAzymes inhibited the expression of $p210^{bcr\text{-}abl}$ protein by 40% in K562 cells and specifically inhibited the growth of *bcr-abl*-positive cells by 53−80%.[95] Since then, the development of DNAzymes advances cancer therapy by modulating cancer cell proliferation/survival,[95−98] angiogenesis,[99,100] ECM degradation,[101,102] and metastasis.[98,102,103]

To improve efficiency and cellular uptake of DNAzymes, physiological barriers, such as cell membranes and degradative enzymes, must be overcome.[104] In one report, CoOOH nanoparticles were used as DNAzyme delivery vehicles to inhibit the progression of cancer cells (Figure 12a).[105] CoOOH was reduced to Co^{2+} by high level of GSH and the Co^{2+} served as cofactor for DNAzyme. Thymidine kinase 1 (TK1), survivin (SUR), and serine-threonine protein kinase AKT1 were selected as target genes, which are responsible for tumor growth, cell division, migration, and apoptosis. The expression level of target mRNAs was reduced by CoOOH-DNAzymes and the cell invasiveness was also inhibited. The intratumor administration of this DNAzyme nanocomposite in mice showed excellent antitumor effects.

Another strategy to increase cellular uptake is incorporating DNAzymes into other DNA sequences to form nanostructures. Zhang and co-workers designed a DNAzyme-integrated DNA nanoflowers (DNFs) fabricated by hybridization of aptamer templates and DNAzymes (Figure 12b).[104] Aptamer AS1411 used as template and early growth response-1 (EGR-1) DNAzyme and survivin DNAzyme were simultaneously incorporated into the aptamer. After rolling cycle amplification, DNFs were formed with dual gene silencing ability to inhibit tumor growth. The uptake of DNFs was cell-selective and tended to accumulate in breast cancer cells but not hepatocytes. Intratumor injections of dual DNFs obviously shrank the tumor volume from ∼600 to 182 mm^3, implying the great potential of DNAzyme system as therapeutics.

3.4. Other Interactions. Other features of TIME, such as acidity and high GSH, though not frequently investigated as ROS and hypoxia, also contribute to immunosuppression. Unlike normal cells, cancer cells utilize glucose by aerobic glycolysis and produce large amounts of lactate, leading to elevated extracellular acidity.[13] The elevated acidity can reduce T cell functions, increase immune checkpoints expression, restrict antigen presentation of dendritic cells, impair the function and proliferation of NK cells, and increase recruitment of immunosuppressive myeloid cells.[106] On the other hand, neutralization of tumor acidity has been proven to be effective in improving antitumor immunotherapy,[107] demonstrating that acidity is a promising target for cancer immunotherapy. Currently, only few reactions catalyzed by nanozymes consume or produce hydrogen ions, which subsequently changes the local pH level. One prominent example is MnO_2 nanoparticle which decompose H_2O_2 into O_2 and consume hydrogen ions ($MnO_2 + 2H^+ Mn^{2+} + H_2O + 1/2\ O_2$; $MnO_2 + 2H^+ + H_2O_2\ Mn^{2+} + 2H_2O + O_2$).[108] On the basis of this principle, to improve CAT activity of MnO_2 nanozyme, a hybrid nanozyme system which incorporates natural glucose oxidase and MnO_2 nanozyme was designed to accelerate O_2 generation and relieve hypoxia for PDT. Glucose oxidase catalyzes glucose into glutamic acid and H_2O_2, thus providing both H^+ and H_2O_2 for subsequent MnO_2-catalyzed reactions.[109] Another study also confirmed the pH-altering ability of MnO_2 nanozyme. Prasad et al. showed that albumin/polyelectrolyte coated MnO_2 nanozyme increased tumor pH from 6.7 to 7.2.[71]

GSH is the most abundant antioxidant and serve as free radical scavenger. In TIME, elevated GSH protects cancer cells from oxidative stress and confer resistance to therapies that produce free radicals.[110] Depletion of GSH can increase the ROS generation which further affect the proliferation and function of immune cells. Lu et al. demonstrated that decreasing GSH can facilitate the proliferation of cytotoxic T lymphocytes.[111] Another study showed that cancer cells overexpressed cystine-glutamate antiporter xCT to fuel the overproduction of GSH. Deletion of xCT in cancer cells led to decreased cystine uptake and GSH production, and combination with anti-CTLA-4 enhanced the frequency and durability of antitumor responses.[112] Cobalt nanoparticle is an exemplar nanozyme with the ability to deplete GSH due to the oxidation of thiol groups in GSH by cobalt.[113] Lately, cobalt-based nanozyme has been adopted to deplete GSH and thus increase ROS generation for enhanced chemodynamic therapy (CDT).[114]

4. APPLICATIONS OF NANOZYMES IN CANCER IMMUNOTHERAPY

Cancer immunotherapy is advantageous over traditional therapies because of the generation of immune memory that can prevent tumor recurrence. However, the immunosuppressive TIME creates a therapeutic barrier to immunotherapy. The intrinsic catalytic properties enable nanozymes to interact with molecular and cellular components of TIME to alleviate immunosuppression. For instance, attenuation of oxidative

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stress and hypoxia can elicit immune responses. In addition, the immunogenic cell death of cancer cells induced by some therapies, such as PDT and RT, can provoke antitumor immune responses via releasing antigens and proinflammatory cytokines.[115] Although nanozymes are widely exploited as cancer therapeutics, their immunological effects are seldom reported. This section highlights the recent applications of nanozymes in cancer immunotherapy. Currently, nanozymes can be used as standalone therapeutics or synergized with other therapeutic strategies for cancer immunotherapy (Table 1).

4.1. Nanozymes as Standalone Therapeutics. As an attempt to adopt DNAzymes for cancer therapy, Khachigian and co-workers developed a DNAzyme-Dz13 as standalone immunotherapeutics for skin cancer treatment.[116,117] Dz13 can target c-Jun, which is one member of the activator protein-1 (AP-1) and involved in regulation of tumorigenesis, including modulating cell proliferation, inhibiting apoptosis, and enhancing angiogenesis and invasiveness.[119] Dz13 contains three unmodified CpG motifs, which can provoke innate immunity via toll-like receptor 9 (TLR-9). In addition to mRNA cleavage, Dz13 could inhibit ROS generation in a myocardial inflammation model because of the inhibitory effect of Dz13 on nitrotyrosine (an indicator of RNS),[120] implying that this nanozyme can modulate immunosuppressive TIME.

In two skin cancer types-basal cell and squamous cell carcinomas, Dz13 inhibited tumor growth, suppressed angiogenesis and induced apoptotic death of cancer cells. Although Dz13 contains CpG motifs, the suppression of squamous cell carcinomas growth by Dz13 was not achieved via activating innate immune responses through modulating TLR-9. Instead, the suppression was mediated by adaptive immunity, with the involvement of $CD4^+$ cells and $CD8^+$ cells. Dz13 may render tumor growth inhibition by either direct cleavage of c-Jun mRNA and suppression of c-Jun-dependent proangiogenic and tumorigenic genes (such as VEGF-A, FGF-2, MMP-2, and MMP-9) or indirectly exposing tumor antigens for recognition and attack by immune systems.[116] Recently, the same group used Dz13 to treat another type of skin cancer-melanoma. Similarly, Dz13 inhibited c-Jun expression and angiogenesis and increased the infiltration of $CD4^+$ T cells into tumors. The authors observed Dz13-mediated prevention of neighboring tumor growth for the first time. Although Dz13 can inhibit the growth of other tumor types, the abscopal effect was only achieved in the melanoma model, probably due to increased $CD4^+$ cells.[117]

4.2. Nanozymes Synergized with Other Therapies. Nanozymes usually coordinate with other therapies to elicit antitumor immunity. To facilitate cancer cell death, $Cu_{2-x}Te$ nanoparticles that exhibited glutathione oxidase- and peroxidase-like activity were synergized with photothermal therapy (PTT) and immune checkpoint blockade therapy (Figure 13).[20,121] The defect-induced near-infrared-II absorption on $Cu_{2-x}Te$ can enhance its catalytic activities and provides photothermal effects. The generated ROS induced immunogenic cell death, which was demonstrated by the release of three markers: adenosine triphosphate (ATP), calreticulin (CRT), and high mobility group box 1 (HMGB-1). In addition to damaging cancer cells, accumulated ROS skewed the polarization macrophage from M2 to M1 phenotype and increased the maturation of dendritic cells. Moreover, more immune cells, such as cytotoxic T lymphocyte (CTL) and T help cells (Th cells) were infiltrated into the tumor site for the

Table 1. Representative Nanozymes Used in Cancer Immunotherapy

nanozyme	enzymatic activities	immune pathways	functions	tumor model	standalone/ synergized therapy	ref
Dz13	cleave mRNA of c-Jun gene	AP-1	inhibit c-Jun and c-Jun-dependent proangiogenic and tumorigenic genes; expose tumor antigens for recognition and attack by immune system; prevent secondary tumors	basal cell and squamous cell carcinoma; melanoma	standalone	116, 117
$Cu_{2-x}Te$	peroxidase and GPx	immunogenic cell death with antigen release	reserve immunosuppressive TIME; boost immunity to eradicate both primary and distant metastatic tumors	4T1 breast tumor	synergized with PTT	20
AuNC@MnO_2	CAT; peroxidase	immunogenic cell death with antigen and DAMPs release	alter immunosuppressive TIME; increase recruitment of T cells; provoke systematic antitumor responses	triple-negative breast tumor	synergized with PDT	118
H-MnO_2–PEG/ Ce6-DOX	CAT	immunogenic cell death and TAA release; PD-L1	relieve hypoxia; shape immunosuppressive TIME into antitumor TIME; increase CTL infiltration	4T1 breast tumor	synergized with PDT and DOX	73
CaO_2/DOX@ SiO_2/DOX-MnO_2	CAT	hypoxia; CD73/CD39; CTLA-4	relieve immunosuppressive hypoxia; sensitize anti-CTLA-4 therapy; enhance the infiltration of $CD8^+$ cells and decreased immunosuppressive Treg cells; DOX elicit the maturation of DCs	B16F10 melanoma tumor	synergized with anti-CTLA-4 and DOX	74

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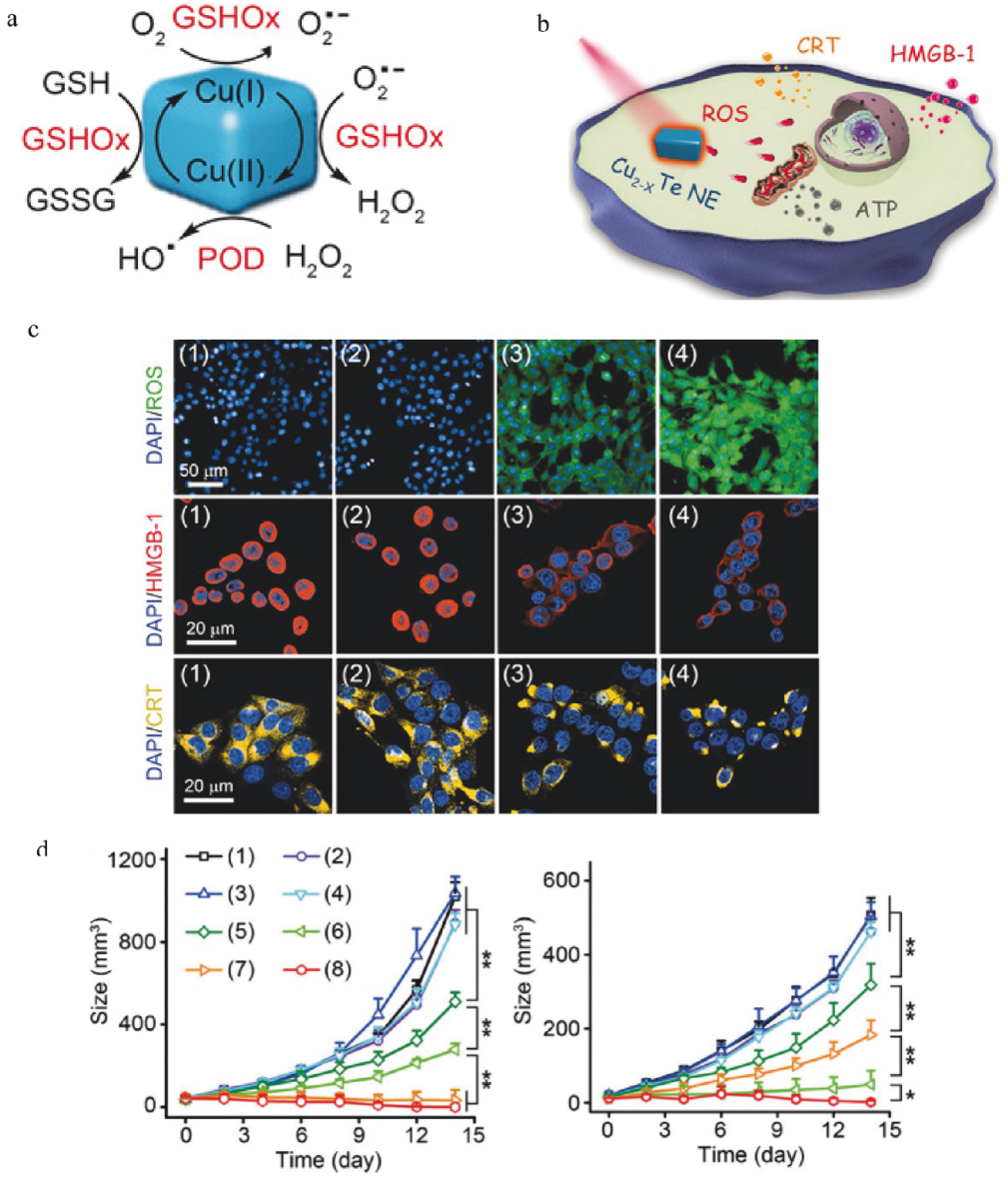

Figure 13. Cu_{2-x}Te nanozyme for catalytic immunotherapy. (a) Proposed cascade reactions catalyzed by Cu_{2-x}Te nanozyme. (b) Scheme of the nanozyme-induced immunogenic cell death under the excitation of NIR-II. (c) Immunofluorescent staining of immunogenic cell death marker-ATP, CRT, and HMGB-1. (d) Combinatory therapeutic effects of anti-PD-L1 and Cu_{2-x}Te nanozyme on primary (left) and distant tumors (right). Cu_{2-x}Te nanozyme boosted antitumor immunity both for primary and distant tumors. Reprinted from ref 20 with permission from John Wiley and Sons. Copyright 2019.

nanozyme-treated group. Combined with anti-PD-L1 antibody, this nanozyme significantly inhibited tumor growth and tumor recurrence in a mouse model.

In a recent study, AuNC@MnO_2 composite nanozyme synergized with PDT for metastatic triple-negative breast cancer (mTNBC) therapy.[118] Gold nanocages (AuNCs) as core were coated with MnO_2 shell and MnO_2 acted as catalase decomposing H_2O_2 to O_2, which could be utilized by PDT. The role of AuNCs played in this composite nanozyme was not clear. As reported previously, ultrasmall gold nanoparticles possess the glucose oxidase-like activity which can convert glucose into gluconic acid and H_2O_2.[122] In this paper, the authors indicated that AuNCs might function as a peroxidase mimic and involve in the photocatalytic decomposition of H_2O_2 for ROS production. The following experiments showed that under NIR excitation, ROS produced by PDT induced the immunogenic cell death with the release of three markers: ATP, CRT, and HMGB-1. The composite nanozyme could be eliminated by major organs after intravenous injection, suggesting the biocompatibility of the nanozyme. In a mice tumor model, under NIR irritation, AuNC@MnO_2 nanozyme could eliminate the tumor, increase the maturation of dendritic cells and the infiltration of immune effector cells, including $CD4^+$ T cells, $CD8^+$ T cells, and NK cells. In addition, this nanozyme-PDT synergized therapy could effectively provoke antitumor immunity and destroy metastatic tumors.

Similarly, MnO_2 nanoparticle was also used as catalase mimic in another study combined with chemo-photodynamic therapy.[73] In this study, hollow MnO_2 (H-MnO_2) nanoparticles were modified with polyethylene glycol (PEG) which coloaded the photosensitizer Ce6 and a chemotherapy drug doxorubicin (DOX). MnO_2 nanozyme decomposed H_2O_2 to

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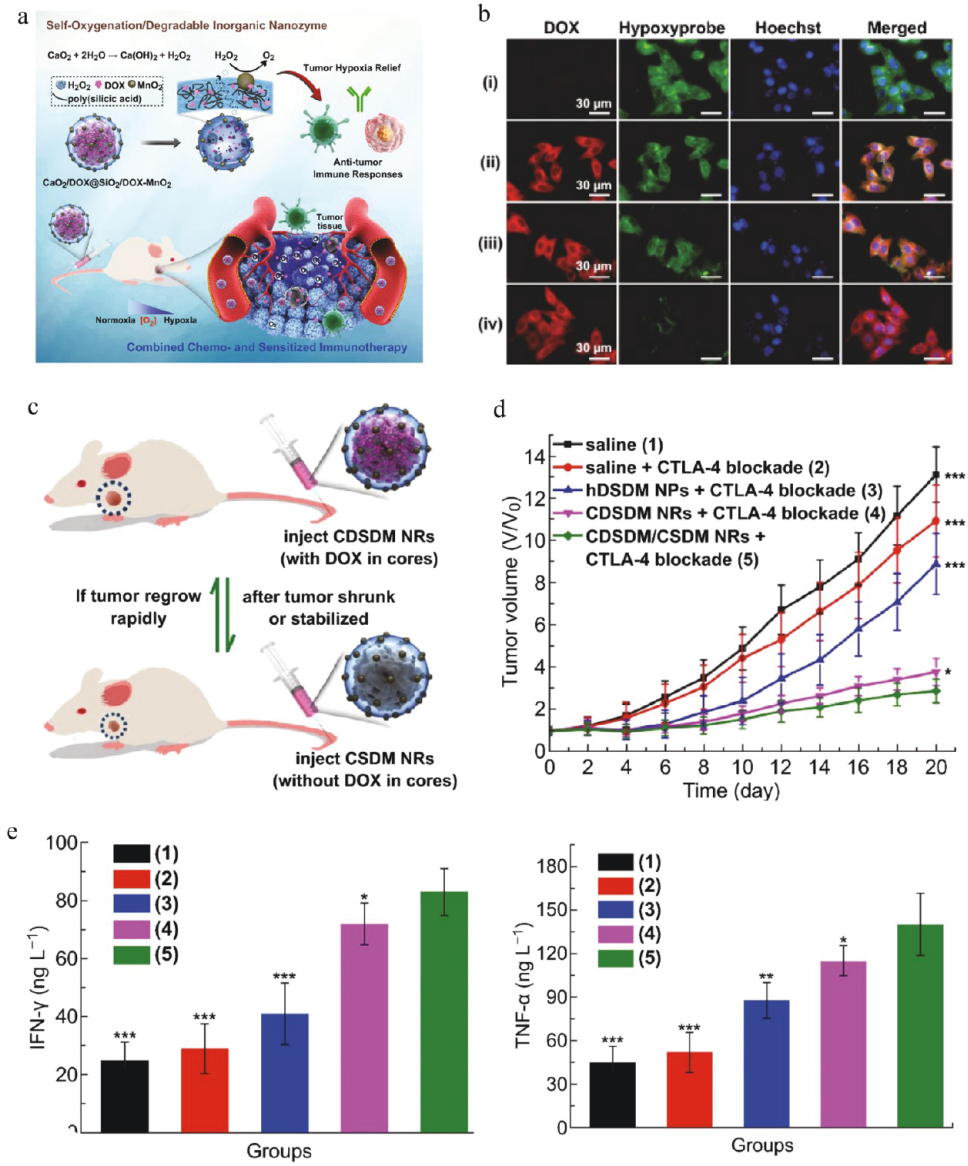

Figure 14. Self-oxygenated nanozyme reactor combined with anti-CTLA-4 for enhanced immunochemotherapy. (a) Scheme of mechanism of this nanozyme reactor for hypoxia relief. (b) Immunofluorescent staining of hypoxia: (I) PBS, (II) HDSDM NPs, (III) CDSD (without MnO_2 nanorods), and (IV) CDSDM NR. (c) Schematic of nanoreactor administration in mice model and tumor volume change after treatments. (d) Proinflammatory cytokines concentration change after administration of nanoreactor combining with anti-CTLA-4. Reprinted from ref 74 with permission from Springer Nature. Copyright 2019.

generate O_2 which further enhanced the therapeutic effects of PDT. This nanoplatform was biocompatible and showed no long-term safety concern in vivo. Combined with immune checkpoint blockade anti-PD-L1, this nanoplatform inhibited tumor growth and elicited antitumor immunity which increased CTL infiltration and TNFα secretion.

Another strategy to improve therapeutic effects is to oxygenate the TIME. Wang et al. designed a nanozyme reactor (NR) with SiO_2/DOX-MnO_2 as the shell and CaO_2/DOX as the core (Figure 14).[74] CaO_2 can be gradually hydrolyzed to produce H_2O_2 which can be decomposed by MnO_2 to relieve the hypoxic TIME. Termed as CaO_2/DOX@SiO_2/DOX-MnO_2 (CDSDM), this nanozyme reactor could effectively relieve hypoxia both in vitro and in vivo. Combined with anti-CTLA-4, this nanozyme system obviously inhibited tumor growth without affecting the weight of mice. In addition, CDSDM NR-anti-CTLA-4 could increase the secretion of proinflammatory cytokines IFN-γ and TNF-α.

5. CONCLUDING REMARKS AND PERSPECTIVES

Cancer therapy, especially immunotherapy, is hindered by the complex TIME comprised of dynamic networks of cells and molecules. With such complexity of TIME, most cancer patients fail to respond to the representative immune checkpoint blockade therapy. Remodeling of TIME can relieve the immunosuppression and provide alternative immunotherapeutic strategies. Nanozymes with unique features, such as catalytic ability, smaller size, ease of fabrication, better tumor penetration, and greater tumor accumulation, are attractive for cancer immunotherapy. Currently, two types of nanozymes-metal/metal oxide nanozymes and DNAzymes can interact with different components of TIME. The intrinsic ROS-targeting property of metal/metal oxide nanozymes usually

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scavenge or generate ROS. While DNAzymes are endowed with the ability to cleave mRNA and therefore, exert their antitumor effects via targeting cellular components of TIME.

ROS-scavenging nanozymes alone can reverse immunosuppressive TIME because of O_2 generation. In addition, ROS-scavenging nanozymes can combine with PDT, SDT, and RT to augment their efficiency mainly by producing ROS to destruct cancer cells. In these combinatory therapies, nanozymes covert local H_2O_2 to O_2, and upon exposure to external energy source and sensitizers, O_2 subsequently is converted to ROS. In contrast, ROS-generating nanozymes can directly induce cancer cell death without synergizing with other therapies. The death of cancer cells provides immunostimulatory components to active immune responses and acquire immune memory. Unlike metal/metal oxide nanozymes, DNAzymes usually target cancer cells and induce cancer cell death. Some in vivo studies observed the involvement of immune cells. Therefore, it is reasonable to speculate that DNAzymes mediate immunogenic cell death which further activate antitumor immune responses. Some DNAzymes require metal ions as cofactors to remain active. This featured property of DNAzymes can couple the therapeutic effects of metal/metal oxide nanozymes. The metal/metal oxide nanozymes provide cofactors for DNAzymes while DNAzymes with the presence of metal ion cofactors, can induce cancer cell death. DNAzyme-conjugated nanoparticles with different targets in TIME will be promising in cancer immunotherapy.

Despite their promising future, nanozyme-based cancer immunotherapy still has limitations and challenges. First, precisely targeting tumor cells is still a challenge for nanozymes. The off-target effect of nanozymes may damage normal cells. In order to reduce the side effects, stimuli-responsive nanozymes, such as pH-responsive and hypoxia-responsive nanozyme systems, should be developed. In addition, typical nanozymes lack specificity and exhibit various enzymatic properties which might impose challenges for their utilization. For example, Mn_3O_4 nanozyme possesses SOD-, CAT-, and GPx-like activities. SOD converts free radicals into H_2O_2 and O_2 while CAT and GPx consume H_2O_2. These multiple enzymatic properties hinder its application in chemodynamic therapy (CDT) since elevated H_2O_2 is crucial for therapeutic efficacy.[114] As enzymatic properties of nanozymes are highly dependent on the local environment, it is important to characterize the enzymatic properties in the context of TIME, such as mildly acidic pH (~6.5) and hypoxia. Lastly, current nanozyme-based cancer immunotherapy mainly focuses on regulation of TIME, especially immunosuppressive molecules and features (such as ROS, acidity, and hypoxia), to enhance immune responses. Novel therapeutic strategies for cancer immunotherapy should also be developed for better treatments.

Until now, nanozymes are still in their infancy and can only mimic very few natural enzymes. With the rapid development of nanotechnology and cancer immunotherapy, nanozymes with higher specificity combined with innovative immunotherapeutic strategies can be expected in the future.

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Notes

The authors declare no competing financial interest.

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