Mn\textsuperscript{3+}-rich oxide/persistent luminescence nanoparticles achieve light-free generation of singlet oxygen and hydroxyl radicals for responsive imaging and tumor treatment

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Abstract

X-ray excited persistent luminescence (XEPL) imaging has attracted increasing attention in biomedical imaging due to elimination of autofluorescence, high signal-to-noise ratio and repeatable activation with high penetration. However, optical imaging still suffers from limited for high spatial resolution.

**Methods:** Herein, we report Mn\textsuperscript{3+}-rich manganese oxide (MnO\textsubscript{x})-coated chromium-doped zinc gallogermanate (ZGGO) nanoparticles (Mn-ZGGOs). Enhanced XEPL and magnetic resonance (MR) imaging were investigated by the decomposition of MnO\textsubscript{x} shell in the environment of tumors. We also evaluated the tumor cell-killing mechanism by detection of reactive oxygen (ROS), lipid peroxidation and mitochondrial membrane potential changes in vitro. Furthermore, the in vivo biodistribution, imaging and therapy were studied by U87MG tumor-bearing mice.

**Results:** In the tumor region, the MnO\textsubscript{x} shell is quickly decomposed to produce Mn\textsuperscript{3+} and oxygen (O\textsubscript{2}) to directly generate singlet oxygen (\textsuperscript{1}O\textsubscript{2}). The resulting Mn\textsuperscript{3+} transforms endogenous H\textsubscript{2}O\textsubscript{2} into highly toxic hydroxyl radical (\cdotOH) via a Fenton-like reaction. The Mn\textsuperscript{2+} ions and ZGGOs also exhibit excellent T\textsubscript{1}-weighted magnetic resonance (MR) imaging and ultrasensitive XEPL imaging in tumors.

**Conclusion:** Both the responsive dual-mode imaging and simultaneous self-supplied O\textsubscript{2} for the production of \textsuperscript{1}O\textsubscript{2} and oxygen-independent \cdotOH in tumors allow for more accurate diagnosis of deep tumors and more efficient inhibition of tumor growth without external activation energy.

Key words: Mn\textsuperscript{3+}-rich oxide, X-ray excited persistent luminescence, tumor environment, chemodynamic therapy, responsive imaging

Introduction

Versus traditional optical technologies, persistent luminescence efficiently avoids the tissue autofluorescence interference due to lack of external illumination from in situ excitation [1, 2]. In particular, near-infrared (NIR) emitting persistent luminescence is a promising candidate for in vivo imaging due to deeper tissue penetration depth and higher signal-to-noise ratio [3, 4]. The excitation source is mainly UV light [5], which cannot repeatedly stimulate the persistent luminescence to realize in vivo imaging.
recent imaging due to a poor tissue penetration depth [6]. Recently, white and red light were used as an excitation source to reactivate persistent luminescence for in vivo imaging [7-9], but the light penetration is still limited for further bio-applications. X-rays have been widely used in deep imaging and therapy in clinic, including as excitation sources for in vivo recharged deep tissue imaging [10-14].

Researchers had demonstrated that ZGGO persistent luminescence nanocomposite could load therapeutic drug for realizing long-term drug tracking and significant tumor therapeutic effect [15, 16]. Moreover, the persistent luminescence materials could be employed as photodynamic therapeutic agents. Recent research showed the ZnGa_{1.996}O_{4}Cr_{0.004} persistent luminescence nanomaterial was an effective excitation source to achieve repeatable photodynamic therapy in vivo and effectively inhibited tumor growth [17]. Our previous study also demonstrated the Zn_{3}Ga_{2}GeO_{8}:Cr^{3+},Yb^{3+},Er^{3+}@mSiO_{2} persistent luminescence nanomaterials realized X-ray induced ultrasensitive persistent luminescence imaging and effective inhibition of orthotopic hepatic tumors [14].

However, persistent luminescence suffers from poor spatial resolution. In contrast, magnetic resonance (MR) imaging offers detailed three-dimensional anatomical images [18]. Thus, multifunctional and/or multi-modal imaging probes can combine these strengths. Manganese oxides have been employed as an excellent choice for cancer diagnostics [19-25]. Manganese oxides (MnO_{x}) can react with the environment in tumor regions, i.e. low pH, overexpressed glutathione (GSH), and high hydrogen peroxide (H_{2}O_{2}) level to release Mn^{2+} and O_{2} [26-32]. Our previous study demonstrated that manganese oxide sweetgum-balls could be used as drug carriers to realize enhanced tumor theranostics [33]. Recent studies showed that MnO_{x} could degrade to produce Mn^{2+} and react with H_{2}O_{2} by generating limited hydroxyl radical (·OH) for chemodynamic therapy (CDT) [34]. CDT is a novel anticancer strategy due to generation of highly toxic reactive oxygen [35, 36]. The Mn^{3+}-contained in MnO_{x} has strong catalytic ability to achieve the light-free generation of 1O_{2} to boost dynamic therapy efficacy [37]. Thus, this Mn approach is a promising strategy to develop nanotheranostics that can generate several kinds of ROS synchronously from multiple reactants for good therapeutic effects.

Scheme 1. Schematic diagrams of Mn^{3+}-rich oxide/persistent luminescence nanoparticles for light-free generation of singlet oxygen and hydroxyl radicals for responsive imaging and tumor treatment.
Herein, we present a facile method to prepare Mn$^{3+}$-rich MnO$_x$ coatings on ZGGO nanoparticles (Mn-ZGGOs) (Scheme 1). The environment in tumor regions stimulated the decomposition of MnO$_x$ coating to release ions and persistent luminescence nanoparticles. Investigations in vitro and in vivo showed that the MnO$_x$ shell could simultaneously release Mn$^{3+}$ to accelerate endogenous O$_2$ into highly toxic $^{1}$O$_2$ and generate more Mn$^{2+}$. The generated Mn$^{2+}$ then transforms endogenous H$_2$O$_2$ into the highly toxic ·OH. Thus, effective tumor inhibition in vivo was attributed to the concurrent generation of two different ROS to achieve parallel CDT. At the same time, the released Mn$^{2+}$ could be used as MR imaging agent with higher spatial resolution. And exposed ZGGOs was also an ultrasensitive XEPL imaging agent with higher tissue penetration depth in tumors.

Methods

Synthesis of Mn-ZGGOs

10 mg ZGGO nanoparticles were dispersed in 10 mL ethanol by sonication for 1 h, and then centrifuged and washed with deionized water. Next, KMnO$_4$ (20 mg) aqueous solution was dropwise added into the suspension of ZGGO under sonication. After 2 h, the precipitate was obtained by centrifugation at 10000 rpm.

In vitro X-ray recharged persistent luminescence imaging

Mn-ZGGOs solution ([Mn] = 0.2 mg/mL, 200 μL) was excited by X-ray (0.1 Gy). After 60 s, the GSH solution (10 mM, 20 μL) was added into Mn-ZGGOs, and XEPL signals with GSH or without GSH were collected on an IVIS Lumina II imaging system in the BLI mode at different time points, respectively. After 2 h, Mn-ZGGOs solution was re-irradiated by X-rays and the recharged images were acquired.

Measurement of ·OH

25 mM NaHCO$_3$ solution containing Mn-ZGGOs NPs and different concentrations of GSH (0, 0.5, 1.0 and 10 mM) was mixed for 30 min. After centrifugation, 10 μg/mL MB and 10 mM H$_2$O$_2$ were added to the supernatant. Then, the mixture was incubated at 37 °C for 30 min, and the absorbance change of MB at 665 nm was measured.

Measurement of $^{1}$O$_2$

Mn-ZGGOs was dissolved in 0.5 mL of PBS with different pH values (7.4, 6.5, and 5.6) and then 5 μL of SOSG (500 μM, dissolved in methanol) was added. After incubation for 30 min, the mixture was centrifuged to remove the unreacted Mn-ZGGOs to avoid the interference of Mn-ZGGOs on SOSG fluorescence.

Generation of O$_2$

Mn-ZGGOs ([Mn] = 0, 0.5 mM) were added into 1 mL PBS solution (pH=5.6) with H$_2$O$_2$ of 10 mM. The produced O$_2$ was detected by the fluorescence change of [Ru(dpp)$_3$]Cl$_2$ probe.

GSH-activated MR imaging performance

The Mn-ZGGOs NPs aqueous solutions with different Mn concentrations were mixed with GSH for 10 min, and then the MRI analysis was performed on 9.4 T clinical MR imaging.

In vitro cytotoxicity

The cytotoxicity of the as-prepared Mn-ZGGOs was investigated using a standard MTT assay. U87MG cells and L02 cells (1 × 10$^4$ cells per well) were seeded into 96-well plates and allowed to grow overnight. The cells were incubated with Mn-ZGGOs at different concentrations for 24 h. After that, 10 μL of MTT was added to each well. After incubation for 4 h, the medium was removed, and 150 μL of DMSO was added to dissolve the emerging formazan crystals. The absorbance at 570 nm was measured with a multi-detection microplate reader.

In vitro generation of ROS

U87MG cells (2 × 10$^5$) were seeded into glass bottom dishes (35 mm × 10 mm) and allowed to grow overnight. Then cells were replenished with fresh medium containing Mn-ZGGOs ([Mn] = 0, 10 and 20 μg/mL). After incubation for 24 h, the cells were washed with PBS for three times and stained with Hoechst 33342 (10 μg/mL) and then 5 μM of DCFH-DA for 20 min at 37 °C. Subsequently, those cells were imaged by Olympus FV1200 laser confocal scanning microscope after washing by PBS.

Intracellular lipoperoxide evaluation

U87MG cells (2 × 10$^5$) were seeded into glass bottom dishes (35 mm × 10 mm) and allowed to grow overnight. The fresh medium containing Mn-ZGGOs ([Mn] = 0 and 20 μg/mL) was added. After incubation for 24 h, the cells were washed with PBS for three times and stained with Hoechst 33342 (10 μg/mL) for 20 min at 37 °C. After washing with PBS for three times, the cells were further stained with SOSG (5 μM) or DCFH-DA (5 μM) for 20 min at 37 °C. Subsequently, those cells were imaged by Olympus FV1200 laser confocal scanning microscope after washing by PBS.
Mitochondrial membrane potential damage evaluation

U87MG cells (2 × 10^5) were seeded into glass bottom dishes (35 mm × 10 mm) and allowed to grow overnight. The fresh medium containing Mn-ZGGOs ([Mn] = 10, 20 μg/mL) was added. After incubation for 24 h, the cells were washed with PBS for three times and stained with Hoechst 33342 (10 μg/mL) for 20 min at 37 °C. After washing with PBS for three times, the cells were stained with JC-1 dye (5 μM) for 20 min at 37 °C. The damage of mitochondrial membrane potential was observed using Olympus FV1200 laser confocal scanning microscope after washing by PBS.

In vitro O_2 generation

U87MG cells (2 × 10^5) were seeded into glass bottom dishes (35 mm × 10 mm) and allowed to grow overnight. The fresh medium containing Mn-ZGGOs ([Mn] = 20 μg/mL) was added. After incubation for 0, 4, 8 and 24 h, respectively, the cells were washed with PBS for three times and stained with Hoechst 33342 (10 μg/mL) for 20 min at 37 °C. After washing with PBS for three times, the cells were stained with [Ru(dpp)3]Cl2 (10 μg/mL) for 20 min at 37 °C. The production of O_2 was evaluated using Olympus FV1200 laser confocal scanning microscope after washing by PBS.

In vivo circulation and biodistribution

Balb/c mice bearing U87MG tumors were intravenously injected with Mn-ZGGOs ([Mn] = 1 mg/kg). The tumors and main organs including hearts, livers, spleens, lungs and kidneys, and blood were collected at varied time (1, 2, 4, 8, 12, 24 and 48 h) after injection and were weighed and digested using HNO3-H2O2 mixture. The Mn content in all samples was measured by ICP-MS.

In vivo XEPL imaging

After X-ray irradiation for 1 min, Mn-ZGGOs ([Mn] = 1 mg/kg) was intravenously injected into U87MG tumor-bearing mice. XEPL images were collected using an IVIS in vivo imaging system and the tumors were activated by X-ray for another 1 min before acquiring XEPL imaging at different times (0, 2, 4, 8, 12 and 24 h) post-injection.

In vivo MR imaging

To confirm the activatable MR imaging effect in tumors, Mn-ZGGOs NPs ([Mn] = 1 mg/kg) were intravenously injected into mice bearing U87MG tumors, and then the MR images were obtained at different time points (0, 2, 4, 8, 12 and 24 h) using small animal MR imaging system (9.4T).

In vivo tumor inhibition

When the tumor size reached to ~ 60 mm³, the U87MG tumor-bearing mice were randomized into various groups (n = 5) with different treatments: (1) PBS, (2) ZGGO (6.3 mg/kg), (3) Mn-ZGGOs (8.9 mg/kg, [Mn] = 1 mg/kg), (4) 2×Mn-ZGGOs (17.8 mg/kg, [Mn] = 2 mg/kg). The mice were intravenously injected with different formulations every third day for four times.

Statistical analysis

All data were presented as mean ± standard deviation. Comparison of the data were conducted with a Student’s t-test (*: P < 0.05, **: P < 0.01, and ***: P < 0.001).

Results and Discussion

Synthesis and characterization

ZGGO nanoparticles (ZGGOs) were prepared following our previous synthesized route [38]. The ZGGOs were then stirred in a mixture of KMnO4 and ethanol to coat MnOx layers on the surface of ZGGOs (Mn-ZGGOs). Transmission electron microscopy (TEM) imaging indicated that uniform layers were successfully formed (Figure 1A). Elemental mapping images revealed that the presence of Zn, Ga, Ge, Cr, Mn and O in Mn-ZGGOs as well as Mn circles confirmed the successful coating (Figure 1B). The MnOx layer had very minor impact on ZGGO emission, which still emitted NIR luminescence at ~ 696 nm under/after X-ray irradiation attributed to the typical 2E → 4A2 transition of Cr^3+ (Figures 1C, S1) [39]. GSH would also be helpful to break up the MnOx layers [40]. Impressively, these XEPL signals could be repeatedly activated by irradiation of X-ray; they recovered the excellent XEPL with GSH treatment (Figures 1D, S2). In addition, to prove the deep tissue-penetration of X-ray excitation and NIR emission, a 1.0 cm pork was placed between solution (Mn-ZGGOs with GSH, Mn-ZGGOs without GSH) and X-ray source (Figure S3). XEPL imaging was performed at 0, 2, and 5 min after ceasing X-ray irradiation. Strong NIR persistent luminescence signals were observed, indicating XEPL possessed deep tissue-penetration depth.

In most previous publications, MnOx nanostructures were mainly employed as carriers for cancer treatment [19, 24, 41, 42]. Interesting, recent studies found that MnOx containing Mn(III) would generate ROS under the intrinsic acidity within tumor [34, 37]. The possible mechanism revealed that the Mn valences (especially Mn(III)) impact the catalytic activity to generate ROS [37]. X-ray photoelectron spectroscopy (XPS) analysis showed the presence of Mn(III).
Mn (II), Mn (III), and Mn (IV); Mn (III) was as high as 46.5 atom% (Figure 1E). Thus, the Mn-ZGGOs would have a high activity to generate ROS.

Responsive O₂ production and MR imaging

We also investigated the decomposition of Mn-ZGGOs under the environment in tumor regions. The color changes with Mn-ZGGOs solution and TEM images indicated the rapid breakup of MnOₓ shell in environment within tumor (acidic, GSH) (Figure S4). The decomposition of MnOₓ would generate Mn²⁺ and O₂ [43]. O₂ was produced in acidic environment (pH=5.6), detecting by the [Ru(dpp)₃]Cl₂ probe [44]. A significant decrease in fluorescence of [Ru(dpp)₃]Cl₂ was shown in acidic environment (pH=5.6) (Figure 1F), indicating that O₂ was generated efficiently under the environment. Obvious brightening signals were observed in T₁-weighted MR images with the presence of GSH versus without GSH, indicating the decomposition and release of Mn²⁺ (Figure S5). The T₁ relaxation rate (r₁) of Mn-ZGGOs with the presence of GSH was 8.3 mM⁻¹·s⁻¹ (9.4T), which was 23.2-fold higher than that without GSH (Figure 1G).

Figure 1. (A) TEM image of Mn-ZGGOs. (B) Elemental mapping images of Mn-ZGGOs. (C) X-ray excited optical luminescence (XEOL) and X-ray excited persistent luminescence (XEPL) spectra of Mn-ZGGOs. (D) XEPL decay images of the Mn-ZGGOs with GSH or without GSH. (E) XPS of Mn 2p for Mn-ZGGOs. (F) Fluorescence spectra of [Ru(dpp)₃]Cl₂ of Mn-ZGGOs with or without H₂O₂. (G) The r₁ value of Mn-ZGGOs with or without 1 mM GSH in 9.4 T MR instrument. (H) UV/Vis absorption spectra and photo (inset) of MB after degradation by H₂O₂ plus GSH-treated Mn-ZGGOs ([HCO₃⁻] = 25 mM, [Mn] = 0.5 mM, [H₂O₂] = 10 mM). (I) Fluorescence spectra of SOSG (5 µM) incubated with Mn-ZGGOs for 20 min in H₂O or different PBS buffer (pH = 7.4, 6.5, 5.6).
Responsive ROS generation

Under simulated environment in tumor regions (H2O2, GSH), the absorbance of methylene blue (MB; OH probe) dropped sharply in the presence of Mn-ZGGOs, indicating the production of OH (Figure 1H). MB degradation efficiency of Mn-ZGGOs reached 51.1% when the GSH concentration was 10 mM, which is higher than that of Mn2+ (34.3%, Figure S6). Moreover, the fluorescence of singlet oxygen sensor green (SOSG) at 525 nm increased sharply under simulated acidic environment when incubated with Mn-ZGGOs, indicating generation of singlet oxygen (Figure 1I). These results were consistent with previous reports, and implied that the high-content of Mn(III) in Mn-ZGGOs could realize oxygen-independent production of OH and 1O2 simultaneously and achieve enhanced chemodynamic therapy (CDT) [37]. Moreover, ZGGOs and Mn-ZGGOs showed excellent colloidal stability in PBS, DMEM, and fetal bovine serum (FBS) for further bioapplications (Figure S7).

In vitro antitumor effect and mechanism

Encouraged by the efficient generation of ROS, we investigated the cellular ROS generation and antitumor effect in vitro. The U87MG cells were incubated with ZGGOs and Mn-ZGGOs, and cell viabilities revealed that the ZGGOs core had no obvious cytotoxicity. The Mn-ZGGOs at low concentrations exhibited excellent cell-killing effect with IC50 of 11.7 μg Mn/mL (Figures 2A, S8). Importantly, Mn-ZGGOs showed low toxicity to normal cells (L02 cells) at the high concentration ([Mn] = 0-50 μg/mL) suggesting less vulnerability to oxidative stress (Figure S9) [45].

We explored the killing mechanism of Mn-ZGGOs. After incubation with Mn-ZGGOs ([Mn] = 0, 10, and 20 μg/mL) for 24 h, U87MG cells were co-stained by Hoechst 33342 (nucleus-staining) and SOSG (1O2 indicator) or DCFH-DA (OH indicator). The U87MG cells were incubated with Mn-ZGGOs and SOSG exhibited strong green fluorescence, indicating the generation of 1O2 (Figure 2B). More obvious green fluorescence was observed in U87MG cells incubated with Mn-ZGGOs and DCFH-DA (Figure 2C). These results demonstrated efficient production of intracellular ROS including 1O2 from reaction of Mn3+ and O2 and OH from the Mn2+-mediated Fenton-like reaction (Figure 2C). More ROS generation induced enhanced cell membrane damage [46]. BODIPY C11 showed significant green fluorescence implying high-levels of lipid peroxidation (Figure 2D).

The mitochondrion is vulnerable to the accumulated ROS in the tumor cell resulting in early cellular apoptosis and changes in the mitochondrial membrane potential [47]. Therefore, we assessed the cell apoptosis by staining with a mitochondrial membrane potential fluorescence probe, JC-1. The red fluorescence decreased and green increased gradually, indicating increased cell damage by ROS (Figure 2E). Furthermore, the decomposition of MnOx would produce O2 and Mn2+ and expose the ZGGOs. Figure 2F shows that the red fluorescence of [Ru(dpp)3]Cl2 gradually decreased as the incubation time increased, indicating continuous O2 supply of Mn-ZGGOs in U87MG cells, this further relieved the tumor hypoxia. In vitro T1-weighted MR imaging of U87MG cells was evaluated by incubation with different concentrations of Mn-ZGGOs. After 24 h, the U87MG cells precipitated to the bottom of the tubes. Versus the control group, significant MR signals were observed in the U87MG cells incubated with Mn-ZGGOs, which showed the concentration-dependent behavior (Figure S10). Moreover, the exposed ZGGOs could recover the excellent XEPL by decomposition of MnOx. Figure S11 shows that the U87MG cells were incubated with Mn-ZGGOs for 24 h and exhibited significantly more enhanced XEPL signals. These results demonstrated that Mn-ZGGOs would generate ROS (1O2, OH) during the decomposition process and release imaging agents (Mn2+, afterglow) as well as release O2 to decrease tumor hypoxia.

In vivo biodistribution and biosafety

The in vivo blood-clearance behaviors and biodistribution were assessed by measuring the concentrations of Mn in dissolved tissues using inductively coupled plasma mass spectrometry (ICP-MS). The accumulation of Mn-ZGGOs in tumor tissue was as high as 13.2 % of the injected dose of Mn element per gram tissue (%ID g-1) at 4 h post-injection (Figure 3A). The pharmacokinetics showed that the half-life of Mn-ZGGOs was 4.5 ± 0.9 h (Figure 3B), which would benefit effective accumulation of Mn-ZGGOs into tumors. Furthermore, the long-term biocompatibility was evaluated by intravenous injection of Mn-ZGGOs ([Mn] = 1 mg/kg) into healthy mice. Blood biochemistry and hematology analysis showed no significant difference in hepatic function, kidney function, and blood indexes before and after injection of Mn-ZGGOs, indicating that Mn-ZGGOs had no systematic toxicity (Figures S12, S13).

In vivo imaging of U87MG tumor

The decomposition of MnOx in vivo was first evaluated by injecting Mn-ZGGOs intratumorally in U87MG tumor-bearing mice as well as normal mice.
subcutaneous tissue (Figure S14, black circle: tumor, red circle: normal subcutaneous tissue, Figure S15). In tumors, obvious T1-MR signals and US signals were observed confirming the release of Mn2+ for MR imaging and generation of O2 for US imaging.

Due to the effective response of the environment in tumor regions, we further studied the tumor-diagnosis of Mn-ZGGOs via in vivo imaging. The Mn-ZGGOs were intravenously injected in U87MG tumor-bearing mice (Mn = 1 mg/kg). Obvious T1-MR signals (red and yellow signals in tumors) were observed and reached a peak at 4 h post-injection, suggesting effective accumulation and respond decomposition of Mn-ZGGOs in U87MG tumors (Figures 3C, S15A); these findings were confirmed with biodistribution analysis.

Figure 2. (A) Viability of U87MG cells after 24 h of incubation with Mn-ZGGOs. (B) Confocal images of U87MG cells stained by SOSG after incubating with PBS or Mn-ZGGOs for 24 h. The green fluorescence indicates the presence of 1O2. (C) Confocal images of U87MG cells stained by DCFH-DA after incubating with PBS or Mn-ZGGOs for 24 h. The green fluorescence indicates the presence of ·OH. (D) Confocal images of lipoperoxides in U87MG cells after incubation with PBS or Mn-ZGGOs for 24 h. The green fluorescence is the lipid ROS after the staining with BODIPY C11. (E) Confocal images of the changes in the mitochondrial membrane potential of U87MG cells after incubation with PBS or Mn-ZGGOs for 24 h. The red fluorescence indicates that the membrane potential is positive, and the green fluorescence indicates that the membrane potential decreases. (F) Confocal images of U87MG cells with production of O2 stained by [Ru(dpp)3]Cl2 after incubating with Mn-ZGGOs versus times. The blue fluorescence from Hochest 33342 indicates the cell nuclei in (b-e). Scale bar: 40 μm.
Figure 3. (A) Biodistribution of Mn-ZGGOs in major organs and tumors after intravenous administration at various time intervals (1, 2, 4, 8, 12, 24, and 48 h). The Mn-ZGGOs concentrations were normalized as the percentage of the injected dose of Mn element per gram of each organ (%ID g⁻¹). (B) Time course of blood levels of Mn-ZGGO levels following intravenous injection. The half-life time (t½) was calculated to be 4.5 ± 0.9 h. (C) In vivo T₁-weighted MR images of mice injected intravenously with Mn-ZGGOs. (D) In vivo XEPL imaging of tumor-bearing mice after intravenous injection of Mn-ZGGOs.

As the ZGGOs core of Mn-ZGGOs also had persistent luminescence property, we then irradiated the tumor regions using X-ray to study the in vivo XEPL imaging after 0, 2, 4, 8, 12, and 24 h post-injection. After X-ray excitation (0.1 Gy), the X-ray excited persistent luminescence (XEPL) gradually increased over time and reached a peak at 12 h post-injection (Figures 3D, S17B). The difference was due to the restoration of persistent luminescence via break-up of the MnOₓ coating in acidic environment in tumor regions.

In vivo U87MG tumor therapy

Following the in vivo study of distribution and decomposition of Mn-ZGGOs, the in vivo anticancer efficacies on U87MG tumor-bearing mice were evaluated. When the tumor volumes reached to 60 mm³, the mice were randomly allocated into four groups (n=5 per group): (1) PBS, (2) ZGGO (6.3 mg/kg), (3) Mn-ZGGOs (8.9 mg/kg, [Mn] = 1 mg/kg), and (4) 2×Mn-ZGGOs (17.8 mg/kg, [Mn] = 2 mg/kg). The tumor size and body weight were monitored every two days after injection. Versus controls, the Mn-ZGGOs groups inhibited tumor growth with the inhibition rate of 41.8 % at an Mn dose of 1 mg/kg (Figure 4A). A significantly enhanced tumor inhibition effect (inhibition rate: 71.8 %) was achieved in the 2-fold Mn-ZGGOs. The typical photographs of tumor tissue and the tumor masses at the end of treatment showed a consistent suppressive trend with tumor growth (Figure 4C, 4D). In addition, hematoxylin and eosin (H&E) staining analysis revealed that the tumor suffered more serious damage in CDT groups than those in PBS and ZGGO groups (Figure 4E). Both body weight and H&E staining of the major organs, including hearts, livers, spleens, lungs and kidneys, had no significant change/damage, indicating that Mn-ZGGOs had minor systemic toxicity (Figures 4B, S18). These results demonstrated that the procedure would produce Mn(III)-rich Mn-ZGGOs, and generate abundant ROS for satisfactory environment-activated chemodynamic therapeutics and imaging agents to with value in guiding precision cancer therapy.
Conclusion

In summary, we report an easy and novel method to prepare Mn(III)-rich nanotheranostics of Mn-ZGGOs. Under the environment in tumor regions (acidic pH, high H$_2$O$_2$ and GSH levels), Mn-ZGGOs could react with the GSH/H$_2$O$_2$ to release the agents for imaging and treatment. The released Mn(III)-pool could directly react with O$_2$ to generate $^1$O$_2$; Mn(II) reacted with H$_2$O$_2$ to generate OH. The released in situ Mn(II), O$_2$ and ZGGOs can be employed as MR, US and persistent luminescence diagnostics to guide precision cancer therapy. The tumor cell-killing mechanism in vitro and in vivo was confirmed systematically by detection of ROS, lipid peroxidation and mitochondrial membrane potential changes and tumor inhibition. Overall, this Mn$^{3+}$-rich Mn-ZGGOs nanotheranostics design promotes the ultrasensitive, X-ray reactivated and high spatial resolution imaging. There is light-free generation of singlet oxygen and hydroxyl radicals for enhanced tumor chemodynamic therapy.

Abbreviations

XEPL: X-ray excited persistent luminescence; MnO$_x$: manganese oxide; ZGGO: chromium-doped zinc gallogermanate; Mn-ZGGOs: manganese oxide-coated chromium-doped zinc gallogermanate nanoparticles; MR: magnetic resonance; ROS: reactive oxygen; O$_2$: oxygen; $^1$O$_2$: singlet oxygen; OH: hydroxyl radical; NIR: near-infrared; GSH: glutathione; CDT: chemodynamic therapy; TEM: transmission electron microscopy; XPS: X-ray photoelectron spectroscopy; MB: methylene blue; SOSG: singlet oxygen sensor green; FBS: fetal bovine serum; ICP-MS: inductively coupled plasma mass spectrometry; H&E: hematoxylin and eosin.

Supplementary Material

Supplementary materials and figures. http://www.thno.org/v11p7439s1.pdf

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Competing Interests
The authors have declared that no competing interest exists.

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