Dimeric Her2-Specific Affibody Mediated Cisplatin-Loaded Nanoparticles for Tumor Enhanced Chemo-Radiotherapy

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Research

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Abstract

**Background:** Solid tumor hypoxic conditions fail to facilitate reactive oxygen species (ROS) generation and formation of DNA double-strand breaks (DSBs) induced by ionizing radiation, ultimately leading to a crucial role in radiotherapy resistance. Recently, there have been significant technical advances in nanomedicine aid to relieve hypoxia by **in situ** production of O$_2$, serving as “radiosensitizer” to induce tumor cells more sensitive to ionizing radiation. However, the off-target damage of surrounding healthy tissues caused by such high-energy radiation is often unavoidable and the tumor cells at some distance from the focal spot of ionizing radiation may avoid damage. Therefore, there is an urgent need to exploit an intelligently targeted nanoplatform to integrate both precisely enhance RT-induced DNA damage and combined therapy.

**Results:** Herein, we developed human epidermal growth factor receptor 2 (Her2)-specific dimeric affibody (Z$_{\text{Her2}}$) mediated cisplatin-loaded mesoporous polydopamine/MnO$_2$/polydopamine nanoparticles (Pt@mPDA/MnO$_2$/PDA-Z$_{\text{Her2}}$ NPs) for MRI and enhanced chemo-radiotherapy of Her2-positive ovarian tumor. These NPs are biodegradable under simulated tumor microenvironment, resulting in cisplatin accelerated release, as well as production of O$_2$. Z$_{\text{Her2}}$ produced by the *E. coli* expression system endowed NPs with Her2-dependent binding ability in the Her2-positive SKOV-3 cells. **In vivo** MRI studies revealed an obvious T$_1$ contrast enhancement at the tumor site. Moreover, these NPs achieved efficient tumor homing and penetration, attributing to the efficient internalization and penetrability of Z$_{\text{Her2}}$. Under X-Ray irradiation, these NPs exhibited the highest tumor growth inhibition effect. Immunofluorescence assay showed these NPs significantly reduced the expression of HIF-1α and improved ROS level, resulting in radiosensitization.

**Conclusions:** The nanocarriers constructed in this study integrated Her2 targeting, diagnosis, RT sensitization, thus providing a new idea for clinical translation in tumor theranostics.

**Background**

Radiotherapy (RT), by precisely operating high energy ionizing radiation at the tumor site, can directly induce tumor cell nuclei DNA break and/or indirectly damage cancer cells by reactive oxygen species (ROS) that produced via water molecule radiolysis in the cells, thus playing an important and indispensable role in various cancer treatments [1, 2]. According to clinical statistics, more than 60% of malignant tumor patients receive RT at a specific stage throughout their illness, and 40% of them can be cured [3, 4]. However, the complex tumor microenvironment (TME), specially solid tumor hypoxic conditions ascribing to an imbalance between supply and consumption of O$_2$ in rapidly proliferating tumor cells as well as dysfunctional tumor vasculature [5], fails to facilitate ROS generation and formation of DNA double-strand breaks (DSBs) induced by ionizing radiation [2, 5], ultimately lead to a crucial role in RT resistance. Furthermore, tumor hypoxic conditions can also induce the upregulation of hypoxia-inducible factor 1α (HIF-1α) which is associated with promotion of endothelial cell survival induced by radiation, and further promote RT resistance [6].
In order to alleviate tumor hypoxic conditions and improve the tumor concentration of O\textsubscript{2} (a resource of radiation-induced ROS) [6], traditional medical methods aim to increase the O\textsubscript{2} content in the tumor site by hyperbaric oxygen inhalation [7, 8]. However, the dysfunctional tumor vascular system hinders the delivery of O\textsubscript{2} to the tumor, and the potential oxygen poisoning, barometric injury, and decompression disease seriously limit its clinical use [9]. On the other hand, for decades, research focused on promoting RT efficacy by improving radiation doses, but the severe side effects on normal tissues and organs caused by excessive high-energy radiation is unavoidable [10, 11]. Recently, there have been significant technical advances in nanomedicine, such as artificial blood substitutes, nanocatalysts aid to relieve hypoxia by intratumoral O\textsubscript{2} delivery or \textit{in situ} production of O\textsubscript{2}, serving as “radiosensitizer” to induce tumor cells more sensitive to ionizing radiation with a low and save dose [11, 12]. For example, Gao \textit{et al.} developed perfluorocarbon (PFC)-based nanoscale artificial red blood cell system to relieve tumor hypoxia and thus realize RT sensitivity. With the red blood cell membrane biomimetic cloaking, these artificial blood cells effectively delivered O\textsubscript{2} into tumor tissue, greatly relieved hypoxia, and thus remarkably improved the antitumor efficacy of RT [13]. In a recent study, Chen \textit{et al.} fabricated a catalase (Cat)-loaded nanoplatform for enhanced RT. The cargo Cat can trigger rapid decomposition of endogenous hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) into O\textsubscript{2} to relieve tumor hypoxia, and thus caused enhanced RT efficacy compared to that of X-Ray radiation alone [14]. Manganese dioxide (MnO\textsubscript{2}) NPs, particularly, present fascinating properties, such as TME-responsive biodegradation, glutathione (GSH)-triggered magnetic resonance imaging (MRI), and serve as a chemodynamic therapy agent, which endows them with great potential for tumor theranostics [15-17]. Moreover, as an inorganic nanocatalyst, MnO\textsubscript{2} possesses the ability to catalyze the generation of O\textsubscript{2} by decomposing H\textsubscript{2}O\textsubscript{2} at the nano level, making MnO\textsubscript{2}-based agents a promising candidate for improving O\textsubscript{2}-dependent therapy efficacy [18-21]. For example, Yang \textit{et al.} developed a biodegradable hollow MnO\textsubscript{2} nanoplatform for TME-specific MRI and drug release as well as modulating hypoxic TME to enhance photodynamic therapy (PDT). Under acid TME, these NPs experienced rapid degradation, resulting in loaded therapeutic molecules (photodynamic agent Ce6, DOX) and the MRI agent Mn\textsuperscript{2+} on-demand release, and simultaneously induced endogenous H\textsubscript{2}O\textsubscript{2} decomposition into O\textsubscript{2} to relieve tumor hypoxia and achieve enhanced PDT efficacy [22].

Despite the superior effectiveness of the achieved MnO\textsubscript{2}-based radiosensitizer reported in recent study, the off-target damage of surrounding healthy tissues caused by such high-energy radiation is often unavoidable [23]. Therefore, there is an urgent need to exploit an intelligently targeted nanoplatform as a magic bullet to precisely enhance RT-induced DNA damage with low side effects. One effective approach is to couple these therapeutic formulations with ligands (such as folate molecule, Arg–Gly–Asp tripeptide, and hyaluronic acid) [24-26] or antibodies [27-29] that specifically recognize tumor cells or tumor vasculature associated antigens. This allows targeted delivery of drugs or radiosensitizers into tumor cells by ligand- or antibody-mediated enhanced endocytosis [30]. However, molecular ligands obtained by chemical approaches have limited affinity for their targets owing to their simple structure, and an increasing number of studies have reported the potential immunogenicity of these synthetic agents, which severely impact their performance [31, 32]. Additionally, the relatively poor thermal or...
chemical stability, poor tumor penetration, slow blood clearance, and expensive preparation of antibodies limits its use to a great extent [33]. Affibody molecules, a class of non-immunoglobulin-derived affinity proteins, consist of 58 amino acids, and can be produced in *E. coli* at high levels and low cost [34]. Attributing to the small molecule weight (only ~6.5 kDa) and three-helical-bundle Z domain, affibody features in reversible and rapid folding rate (the folding time is only 3 μs), high thermal tolerance, high specificity and nanomolar affinities of tumors [34]. Meanwhile, the robust molecular structure endows the affibody with high chemical tolerance, including a wide range of pH (5.5–11) [35]. Affibodies antagonizing human epidermal growth factor receptor 2 (Her2), which is specifically overexpressed in a significant number of ovarian, gastric and breast cancers, were well developed and applied in recent years [36-38]. Among all these Her2-specific affibodies, the new generation Z\textsubscript{Her2:2891} having higher thermal and chemical stability as well as hydrophilicity was conjugated with a DOTA moiety (denoted ABY-025) at a unique C-terminal cysteine and has been launched a series of molecular imaging clinical trials [36, 37]. Moreover, the excellent tissue penetration and rapid renal clearance compared to that of antibodies [35], makes affibody meet the requirements for targeted coupling on therapeutics. However, the organic integration between affibody and nanoplatforms is rarely reported in previous literature.

Since polymerization might improve the stability and avidity of affibody [39], in this study, we thus prepared the dimeric Z\textsubscript{Her2:2891} affibody (Z\textsubscript{Her2}) by the *E. coli* expression system and then couple the Z\textsubscript{Her2} to cisplatin (Pt)-loaded sandwich-like nanoparticles (NPs) for MRI and enhanced chemoradiotherapy of Her2 overexpressed ovarian cancer. As displayed in Scheme 1, mussel-inspired mesoporous polydopamine nanoparticles (mPDA NPs), characterized with high biocompatibility, easy face functionalization and wet adhesion [40, 41], were first prepared by the nanoemulsion assembly method. The tumor cells at some distance from the focal spot of ionizing radiation may avoid damage, and to address this challenge, we used the mPDA NPs to deliver cisplatin (Pt) for the chemo-radiotherapy combination. After loading cisplatin, a thin MnO\textsubscript{2} layer was grown on the peripheral surface of Pt@mPDA NPs by *in situ* reduction of KMnO\textsubscript{4} to induce endogenous H\textsubscript{2}O\textsubscript{2} decomposition into O\textsubscript{2} to relieve tumor hypoxia, and thus realize radiosensitization. To improve the biocompatibility of the NPs and provide a reactive surface, a biomimetic PDA layer was polymerized on the surface of the MnO\textsubscript{2} layer, yielding Pt@mPDA/MnO\textsubscript{2}/PDA NPs. The Z\textsubscript{Her2} was ultimately linked to the peripheral PDA layer, giving the intelligent Pt@mPDA/MnO\textsubscript{2}/PDA-Z\textsubscript{Her2} NPs for tumor-specific MRI guiding enhanced combined therapy. These formulations were well characterized in detail, and their precise antitumor efficacy was comprehensively explored both *in vitro* and *in vivo*.

**Materials And Methods**

**Materials**

Pluronic F127 (Mw: 12.6 kDa) was obtained from Sigma-Aldrich (USA). Dopamine hydrochloride (DA·HCl), 1,3,5-trimethylbenzene (TMB), dimethyl sulfoxide (DMSO), cisplatin (Pt, 65%), and cyanine-5.5 (Cy5.5) were purchased from Aladdin (Shanghai, China). Ammonium hydroxide (25.0–28.0%), ethanol,
potassium permanganate (KMnO₄), and hydrogen peroxide (H₂O₂, 30% v/v) were procured from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Isopropyl-β-d-thiogalactoside (IPTG), 2-Mercaptoethanol (2-ME), 6-Carboxyfluorescence (6-FAM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyldiazonium bromide (MTT), glutathione (GSH), 2,7-dichloro-7-hydroxyflourescein diacetate (DCFH-DA), calcein-AM, and propidium iodide (PI) were sourced from Sigma-Aldrich (USA). High-glucose Dulbecco's modified Eagle medium (DMEM), fetal bovine serum, penicillin and streptomycin, trypsin-ethylenediaminetetraacetic acid (trypsin-EDTA), and phosphate-buffered saline (PBS) were obtained from Gibco (Carlsbad, CA). Tris(hydroxymethyl)aminomethane buffer (Tris-buffer, pH 8.6), McCoy's 5A medium supplemented with penicillin (100 U/mL) and streptomycin (100 U/mL) were acquired from Jiangsu KeyGEN BioTECH Co., Ltd (Nanjing, China). Human umbilical vein endothelial cell (HUVEC), breast cancer cell line MCF-7, and Her2-positive human ovarian cancer cell line SKOV-3 were provided by the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Deionized (DI) water (>18.2 MΩ·cm) used for all experiments was purified using a Millipore system, and all chemicals were used without further purification.

**Preparation of Pt@mPDA/MnO₂/PDA NPs**

Mesoporous polydopamine nanoparticles (mPDA NPs) were synthesized according to a nanoemulsion assembly approach [47] with some slight modifications. In details, 2.0 g of Pluronic F127 and 1.0 g of DA·HCl were dissolved in 200 mL of 50% v/v ethanol, and stirred at 1000 rpm for 3 h. Then, 1 mL of TMB was added dropwise to the mixture and sonicated for 5 min to allow generation of nano-emulsion. After stirring for another 30 min at 500 rpm, 10 mL of NH₄OH was added to the resultant mixture while stirring under aerobic conditions to induce the self-polymerization of dopamine. After another 3 h of continuous reaction, and then centrifugation, the mPDA NPs were collected and washed thoroughly with absolute ethanol, and then dispersed in PBS for further use. For drug loading, 200 μL of DMSO containing cisplatin (25 mg) was added to the mPDA NPs (50 mg, 50 mL in PBS), followed by sonication for 30 min, and stirred for 24 h in the dark. The cisplatin-loaded mPDA NPs (Pt@mPDA NPs) were collected after centrifugation and washed with DI water.

The Pt@mPDA NPs were re-dispersed into DI water (50 mL; pH 7.4), and 50 mg of KMnO₄ was added while stirring at 400 rpm for 6 h. After sonication for another 6 h, the mixture was centrifuged and washed thoroughly with DI water, yielding Pt@mPDA/MnO₂ NPs. Finally, the obtained Pt@mPDA/MnO₂ NPs were re-dispersed in Tris-buffer (100 mL; pH = 8.6) and 50 mg of DA was added. The mixture was stirred (400 rpm) for 4 h, and then washed with DI water. This yielded Pt@mPDA/MnO₂/PDA NPs which was re-dispersed into DI water for further use.

**Preparation and characterization of Z_Her2 affibody**

Z_Her2 affibody was expressed and purified according to the method of our previous work [48] with some modifications. In details, a gene encoding the anti-Her2 affibody molecule [34] with adding a cysteine on the C-terminus was synthesized by GenScript (Nanjing, China) and cloned into pQE30 at the BamHI and
SalI sites to construct the expression plasmid pQE30-Z_{Her2}. The plasmid was transformed into *E. coli* M15 and induced overnight with 0.05 mM IPTG at 28 °C. Subsequently, the cells were collected by centrifugation, resuspended in lysis buffer (50 mM phosphate, pH 8.0, 300 mM NaCl, and 20 mM imidazole) and then sonicated on ice for 30 min to lyse the cells. The recombinant proteins in the supernatant were purified using Ni-NTA affinity chromatography according to the manual provided by the manufacturer (GenScript, Nanjing, China). The samples collected during the Z_{Her2} preparation process were detected using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed using Image J software (Bethesda, MD, USA). The purified affibody was dialyzed against phosphate-buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_{2}HPO_{4}, and 1.4 mM KH_{2}PO_{4}, pH 7.4) and then quantified using the Bradford Protein Assay Kit (Beyotime, Jiangsu, China).

The aggregation form of Z_{Her2} was detected using SDS-PAGE with or without 2-Mercaptoethanol (2-ME) in the loading buffer. For the specific cell binding assay, Z_{Her2} was labelled with 6-FAM (Ruixi Biology, Xi’an, China) according to the manufacturer’s protocol: 10 μL of 6-FAM (10 mg/mL; in DMF) was added to 5 mL of Z_{Her2} solution (2 mg/mL; pH 8.3, in PBS). The reaction continued for 1 h at 25 °C in the dark before the mixture was dialyzed against PBS (pH 7.4) at 4 °C for 48 h to remove unreacted 6-FAM. The conjugation of 6-FAM to the Z_{Her2} affibody was verified by SDS-PAGE.

**Conjugation of Z_{Her2} affibody to NPs**

The Pt@mPDA/MnO_{2}/PDA-Z_{Her2} NPs were constructed by conjugating Z_{Her2} to Pt@mPDA/MnO_{2}/PDA via a Michael addition/Schiff base reaction between the amino group and the oxidized quinone form of catechol groups at weak alkaline pH conditions [49, 50]. The chemical stability of Z_{Her2} in reactive Tris-buffer (pH 8.6) was first evaluated before reaction. Two equal amounts of Z_{Her2} were diluted in PBS (pH 7.4) or Tris-buffer (pH 8.6), respectively. The samples were incubated at 37 °C while stirring. At predetermined time points, 1 mL of sample was collected and measured using a UV-Vis spectrometer.

Subsequently, Pt@mPDA/MnO_{2}/PDA NPs were dispersed in Tris-buffer (100 mL; 0.5 mg/mL), and 1 mL of Z_{Her2} solution (1 mg/mL; in PBS) was added into the dispersion followed by sonication (40 kHz; 70 W) for 30 min. Then, the mixture was stirred overnight at 25 °C. The unreacted Z_{Her2} was removed by centrifugation and thoroughly washed with DI water. This yielded Pt@mPDA/MnO_{2}/PDA-Z_{Her2} NPs which was re-dispersed in PBS for further use.

**Characterization techniques**

The morphologies of the NPs were observed by transmission electron microscopy (TEM, JEOL 2010F) at an accelerated voltage of 200 kV. The surface area and pore size of the mPDA NPs were measured using an automated surface area and porosity analyzer (Quantachrome, Autosorb-iQ). X-ray photoelectron spectroscopy (XPS) was performed using a Thermo Fisher ESCALAB 250Xi spectrometer to determine the chemical state of MnO_{2}. Zeta potential and particle size distribution were measured using a Malvern Zetasizer (Nano-ZS, Malvern, UK). UV-Vis absorbance spectra were recorded on a UV-2100...
spectrophotometer. The concentrations of MnO$_2$, cisplatin and Z$_{H_{er2}}$ in the NPs were analyzed by measuring Mn, Pt and S element using inductively coupled plasma-atomic emission spectrometry (ICP-AES, Prodigy, LEEMAN).

**Biodegradation and in vitro drug release**

Pt@mPDA/MnO$_2$/PDA NPs were dispersed in PBS (pH 7.4) supplemented with (1) 1 mM H$_2$O$_2$, (2) 1 mM H$_2$O$_2$, 2 mM GSH, (3) 1 mM H$_2$O$_2$, 5 mM GSH, and incubated at 37 °C while shaking (120 rpm). At each expected time point, 1 mL of each sample was taken for UV-Vis absorbance measurement. After 2 weeks, each sample (200 μL) was added into 24-well plates in triplicate and imaged with a digital camera. Subsequently, the residual samples were centrifugated and re-dispersed in ethanol. TEM images were collected to determine the extent of degradation.

The concentration of the drug release from the NPs was measured according to the dialysis method of our previous work [15] with some modifications: 3 mg of Pt@mPDA/MnO$_2$/PDA NPs was dispersed in PBS (2 mL; pH 7.4), and then loaded into a dialysis bag (MWCO = 7000 Da) and immersed in 18 mL of PBS containing: (1) 1 mM H$_2$O$_2$, pH 7.4; (2) 1 mM H$_2$O$_2$, 5 mM GSH, pH 7.4; (3) 1 mM H$_2$O$_2$, 5 mM GSH, pH 5.5. All samples were incubated at 37 °C while shaking (120 rpm) for 2 days. At predetermined time points, 1 mL of external medium was extracted and supplemented with an equal volume of fresh pre-heated medium. The concentration of the released cisplatin was determined quantitatively by ICP-AES. All experiments were evaluated in triplicate, and the data was presented as mean ± standard deviation (S.D.), n = 3.

**Measurement of dissolved O$_2$**

The ability of MnO$_2$-based NPs to catalyze the decomposition of H$_2$O$_2$ to O$_2$ was measured using a dissolved oxygen meter (JPSJ-605, INESA). Pt@mPDA/MnO$_2$/PDA NPs were dispersed in PBS ([MnO$_2$] = 2 μg/mL) and then transferred into a double-neck flask. H$_2$O$_2$ (30% w/v) was added to the dispersion at a final concentration of 1 mM. A blank PBS or PBS containing H$_2$O$_2$ was used as a control medium. The concentration of dissolved O$_2$ was measured by the probe at predetermined time points.

**GSH-triggered T$_1$-weighted MRI**

Pt@mPDA/MnO$_2$/PDA NPs with varied concentrations ([Mn] = 1 mM, 2 mM, 3 mM, 4 mM, and 5 mM; in PBS) were each treated with 0 or 2 mM GSH. After 20 min, the T$_1$-weighted relaxation times were measured using a 0.5 T NMI20 NMR Analyzing and Imaging system (Niumag, Shanghai, China) at 25 °C. The test parameters were the same as those reported in a previous work [15]. The T$_1$ relaxivity ($r_1$) was acquired through a linear fitting of 1/T$_1$ as a function of Mn concentration. In addition, T$_1$ MRI was performed for samples at different concentrations using a clinical MR system (1.5 T, SIEMENS MAGNETOM Symphony).
**In vitro cellular uptake evaluation**

The expression of Her2 in the breast cancer cell line MCF-7 and human ovarian cancer cell line SKOV-3 was evaluated. MCF-7 and SKOV-3 cells were incubated in DMEM or McCoy’s 5A medium supplemented with 1% penicillin, 1% streptomycin, and 10% fetal bovine serum. The cells were cultured at 37 °C in a 5% CO₂ humidified atmosphere. Afterwards, MCF-7 cells and SKOV-3 cells (2 × 10⁵) were digested and resuspended in DMEM or McCoy’s 5A medium containing 2 or 4 μg/mL FITC-anti-Her2 antibody (Sino Biological, Beijing, China) at 37 °C for 2 h. After incubation, the cells were washed three times with PBS and resuspended in PBS (0.5 mL). The FITC fluorescence intensity was determined using a Becton-Dickinson FACScan analyzer (Franklin, CA, USA). Three independent experiments were conducted. The visualization of the distribution of FITC-anti-Her2 antibody in cells was further analyzed by confocal laser scanning microscopy (CLSM, Carl Zeiss LSM 700). Specifically, MCF-7 or SKOV-3 cells were seeded into 24-well plates and cultured at 37 °C. After 12 h, two types of cells were incubated with 2 or 4 μg/mL FITC-anti-Her2 antibody for another 2 h. The cells were washed three times with PBS, fixed with 4% paraformaldehyde for 15 min at 4 °C, and then washed again three times with PBS. Finally, the cells were immediately observed using CLSM.

The specific affinity between Z_Her2 and Her2-positive cancer cell lines was evaluated. Her2-negative MCF-7 cells or Her2-positive SKOV-3 cells were seeded in a confocal dish (5 × 10⁴ cells per dish) and incubated for 12 h. After that, the medium was aspirated and replaced with 2 mL fresh medium containing 50 μg/mL FAM-Z_Her2. After another 2 h of incubation, the cells were washed three times with PBS, and then fixed with 4% paraformaldehyde for 15 min at 4 °C. The cell nuclei were stained with DAPI (1 mL; 10 μg/mL) for 5 min, and then washed three times with PBS. Finally, the cells were immediately observed using CLSM. The affinity between FAM-Z_Her2 and SKOV-3 cells was further evaluated by flow cytometry (FCM) assay. Approximately 2 × 10⁵ digested cells were incubated with FAM-Z_Her2 (50 μg/mL) at 37 °C for 2 h. For Her2 receptor blocking experiments, SKOV-3 cells were pre-incubated with Z_Her2 (20 μg/mL) for 1 h, and then incubated with FAM-Z_Her2 (50 μg/mL) for another 2 h. After that, the cells were washed three times with PBS and re-dispersed in 0.5 mL PBS for FCM assay.

In order to evaluate the affinity of monomer and dimer of the affibody to Her2 receptor, flow cytometry analysis was done. Briefly, SKOV-3 cells (2 × 10⁵) were digested and incubated with the same molar concentration (100 nM) of monomer (in the presence of 2-ME) or dimer (in the absence of 2-ME) FAM-Her2 affibody at 37 °C for 2 h. After incubation, the cells were washed three times with PBS and re-dispersed in PBS (0.5 mL). The FAM fluorescence intensity was determined by flow cytometry.

To visually track the distribution of the NPs in cells, Cy5.5, instead of Pt, loaded NPs (Cy5.5@mPDA/MnO₂/PDA or Cy5.5@mPDA/MnO₂/PDA-Z_Her2 NPs) were prepared following the same method as described above. The SKOV-3 cellular uptake of these NPs was evaluated following a protocol similar to that described above, except that an FBS-free medium containing Cy5.5@mPDA/MnO₂/PDA or Cy5.5@mPDA/MnO₂/PDA-Z_Her2 NPs (50 μg/mL) was added after the initial culture. To investigate Her2-
dependent binding, SKOV-3 cells were pre-incubated with free Z\textsubscript{Her2} (20 μg/mL) for 1 h prior to the incubation with Cy5.5@mPDA/MnO\textsubscript{2}/PDA-Z\textsubscript{Her2} NPs (50 μg/mL). Finally, the cells were treated and probed by CLSM as described above.

FCM analysis was performed to semi-quantify the uptake of these two NPs by SKOV-3 cells. In detail, about 2 × 10\textsuperscript{5} cells were incubated with Cy5.5@mPDA/MnO\textsubscript{2}/PDA or Cy5.5@mPDA/MnO\textsubscript{2}/PDA-Z\textsubscript{Her2} NPs (50 μg/mL) after a 1 h pre-treatment with or without free Z\textsubscript{Her2} (20 μg/mL). After 4 h incubation at 37 °C, the cells were washed three times with PBS and re-dispersed in 0.5 mL PBS for FCM assay.

**Cytotoxicity assays**

The cytocompatibility of drug-free mPDA/MnO\textsubscript{2}/PDA-Z\textsubscript{Her2} NPs to HUVECs was first studied by MTT assay. HUVECs (~1 × 10\textsuperscript{4}) were seeded into each well of 96-well plates and cultured overnight at 37 °C in a 5% CO\textsubscript{2} humidified environment. The medium was replaced with fresh DMEM containing varied concentrations of mPDA/MnO\textsubscript{2}/PDA-Z\textsubscript{Her2} NPs (1, 5, 10, 20, 50, 100, and 250 μg/mL), and the cells were incubated for another 24 h. Subsequently, the medium of each well was carefully discarded followed by the addition of MTT solution (20 μL; 10 μg/mL), and the cells were incubated for an additional 4 h. Finally, 200 μL of DMSO was added after removing the medium, and the absorbance of the wells at 570 nm was measured with a microplate reader (Multiskan FC, Thermo Scientific).

The in vitro anticancer efficacy of different cisplatin formulations was investigated using a method similar to that described above, except that media containing free cisplatin, Pt@mPDA/MnO\textsubscript{2}/PDA NPs, or Pt@mPDA/MnO\textsubscript{2}/PDA-Z\textsubscript{Her2} NPs ([cisplatin] = 0.6, 3, 6, 12, 24, 48 μg/mL) was added after the initial incubation. Data are reported as mean ± S.D., with three independently performed experiments each containing three replicates. The cells treated with free ciaplatin, Pt@mPDA/MnO\textsubscript{2}/PDA NPs or Pt@mPDA/MnO\textsubscript{2}/PDA-Z\textsubscript{Her2} NPs ([cisplatin] = 48 μg/mL) for 24 h were further stained with PI (staining dead cells red) and calcein-AM (staining live cells, green) in PBS solution for 30 min at 37 °C in the dark, and then imaged by inverted fluorescence microscopy (Carl Zeiss). For all experiments, PBS-treated cells were used as control.

**Analysis of intracellular ROS levels**

The levels of intracellular ROS were probed using DCFH-DA to evaluate the RT-sensitized effect triggered by MnO\textsubscript{2}. SKOV-3 cells were seeded onto 24-well plates at 50,000 cells/well and maintained in McCoy's 5A medium. After 12 h incubation at 37 °C, the cells were treated with (1) PBS, (2) PBS + X-Ray, (3) MnO\textsubscript{2}-free mPDA/PDA-Z\textsubscript{Her2} NPs (50 μg/mL) + X-Ray, (4) mPDA/MnO\textsubscript{2}/PDA-Z\textsubscript{Her2} NPs (50 μg/mL), and (5) mPDA/MnO\textsubscript{2}/PDA-Z\textsubscript{Her2} NPs (50 μg/mL) + X-Ray, respectively. After incubation for 4 h, the cells were irradiated with X-Ray (6 Gy). Another 1 h later, DCFH-DA solution (1 mL; 10 μM) was added to the cells for another 30 min. Subsequently, the fluorescence images were acquired by an inverted fluorescence microscopy (Carl Zeiss).
Animals and tumor model

All animal experiments were carried out with full authorization approved by the ethical committee for animal care of Shandong Cancer Hospital and Institute Shandong, First Medical University and Shandong Academy of Medical Sciences (Approval No. SDTHEC2020004083). Female BALB/C nude mice (SPF grade, 4–6 weeks old) were acquired from Beijing Huafukang Bioscience Co. Inc. (Beijing, China). Tumors were established by subcutaneous injection of SKOV-3 cells ($1 \times 10^6$) dispersed in 100 μL of PBS into the right flank of each mouse. The tumor volume was monitored in real-time and calculated as length × width$^2$/2.

In vivo MRI and biodistribution

Aiming to demonstrate the TME-triggered MRI ability of the MnO$_2$ layer, 50 μL of PBS containing Pt@mPDA/MnO$_2$/PDA NPs ([Mn] = 50 mM) was indirectly injected into the tumor sites or the muscle on the opposite side. At pre-expected time points (0 min, 5 min, and 120 min), the SKOV-3 tumor-bearing mice were scanned using an MR analysis and imaging system (1.5 T, SIEMENS MAGNETOM Symphony).

When the tumor volume reached approximately 200 mm$^3$, Pt@mPDA/MnO$_2$/PDA NPs or Pt@mPDA/MnO$_2$/PDA-Z$_{-\text{Her}2}$ NPs ([Mn] = 3 mM, 100 μL in PBS per mouse) were intravenously injected into the tumor-bearing mice, and T$_1$-weighted MR images were obtained at different time points after injection. The following MR scanning parameters were set: TE = 16.9 ms, TR = 760 ms, FOV = 10 cm × 10 cm, slice thickness = 3 mm, and point resolution = 512 mm × 512 mm.

The mice were executed at 12 h, and the heart, liver, spleen, lung, kidney, brain, muscle, and tumors were extracted and weighed, and then dissolved in aqua regia solution (2 mL; 65 °C) for 24 h. Finally, the Pt content present in different organs and tumors was quantified by ICP-AES.

Immunofluorescence and bio-TEM assays

The SKOV-3 tumor-bearing mice were intravenously injected with 100 μL of PBS containing Cy5.5@mPDA/MnO$_2$/PDA NPs or Cy5.5@mPDA/MnO$_2$/PDA-Z$_{-\text{Her}2}$ NPs (1 mg/mL). For the immunofluorescence assay, tumors were collected after 12 h post-injection and fixed with OCT (Sakura) for further sections under frozen conditions. The tumor sections were incubated overnight with rabbit anti-mouse Her2 primary antibody (dilution 1:200, Abcam) against Her2 at 4 °C, and then for 60 min with goat anti-rabbit secondary antibody (dilution 1:200, Abcam) at 37 °C. The cell nuclei were stained with DAPI. Finally, the obtained slices were scanned using an imaging system (Nikon DS-U3).

For bio-TEM observation, tumors were treated with 1% OsO$_4$ for 2 h at 25 °C, and embedded in resin after the cells were dehydrated. Then, ultrathin sections (70–90 nm) of tumor tissues were cut and further studied by bio-TEM (Hitachi HT7700, Tokyo, Japan).

In vivo antitumor efficacy and safety evaluation
When the volume of tumors reached ~50 mm$^3$, the tumor-bearing mice were randomly divided into four groups (n = 5) and intravenously injected with (1) PBS; (2) free cisplatin; (3) Pt@mPDA/MnO$_2$/PDA NPs; and (4) Pt@mPDA/MnO$_2$/PDA-Z$_{Her2}$ NPs (dose of cisplatin = 2 mg/kg) every two days. Tumor volume and body weights were monitored and recorded every day since the first injection. On the 14$^{th}$ day, 0.5 mL of blood from each group of mice (n = 3) was withdrawn for biochemistry, after which the experiment was halted. The tumors were collected and stained with terminal deoxynucleotidyl transferase UTP nick end labelling (TUNEL) for the apoptosis assay, while the major organs (heart, liver, spleen, lung, and kidney) were extracted and stained with haematoxylin and eosin (H&E) for histological analysis.

For chemo-radiation combined therapy, when the volume of tumors reached ~50 mm$^3$, four groups of tumor-bearing mice (n = 5) were treated with (5) PBS, (6) PBS + X-Ray, (7) MnO$_2$-free Pt@mPDA/PDA-Z$_{Her2}$ NPs + X-Ray; and (8) Pt@mPDA/MnO$_2$/PDA-Z$_{Her2}$ NPs + X-Ray. All groups of mice except for group (5) received cisplatin and MnO$_2$ doses of 2 mg/kg and 3.4 mg/kg, respectively, every two days. For radiotherapy, mice received an X-Ray radiation at a dose of 6 Gy for 24 h post-injection every four days. Tumor sizes and body weights were recorded every day. After 14 days, all the mice were sacrificed, and tumors were collected for volume measurement and weighting. Finally, to evaluate tumor hypoxia levels, the tumor tissues from groups (1)-(4) and group (7) were HIF-1$\alpha$ stained according to the procedure provided by the manufacturer.

Statistical analysis

All results are presented as mean ± standard deviation (S.D.), and the significance of the data was determined using one-way ANOVA. A p value < 0.05 indicates statistical significance, and data are represented as (*) for p < 0.05, (**) for p < 0.01, and (***) for p < 0.001. NS was considered to be no statistically significant.

Results And Discussion

Preparation and characterization of Pt@mPDA/MnO$_2$/PDA NPs

Mesoporous PDA (mPDA) NPs were first synthesized using a nanoemulsion assembly approach. Transmission electron microscopy (TEM) images show that the as-made mPDA NPs are highly uniform with mesostructured morphologies (Fig. 1a), and appear to have a mean size of 119 nm. The mesoporous shape was further characterized by N$_2$ absorption–desorption. As shown in Fig. S1a and 1b (Supplementary Materials), the surface area and average pore diameter of the mPDA NPs were 36.84 m$^2$/g and 2 nm, respectively. After cisplatin loading by electrostatic force between negative-charged mPDA and positive-charged cisplatin (Fig. S1c), attributed to the redox reaction between reductive PDA and KMnO$_4$ under neutral conditions, a MnO$_2$ layer was grown on the surface of Pt@mPDA NPs, where a thin layer was clearly observed at the exterior of the NPs (Fig. 1b), and the mean size increased to 139 nm. For the purpose of improving the biocompatibility, as well as providing a chemically reactive surface for further functional modification, a biomimetic PDA layer was then polymerized on the surface of
Pt@mPDA/MnO$_2$ NPs in a weak alkaline condition (pH 8.6). This is shown in Fig. 1c: the mean size further increased to ~163 nm, and the mesoporous structure almost disappeared.

Step-wise Zeta potential changes (Fig. S1c) were observed, indicating the successful coating of the MnO$_2$ or PDA layer on the NPs. After the final PDA layer surface functionalization, this yielded Pt@mPDA/MnO$_2$/PDA NPs.

The chemical state of the Mn-derived NPs was further determined. The wide-scan X-ray photoelectron spectroscopy (XPS) spectra (Fig. S2a) of the NPs show the characteristic peaks of C 1s, N 1s, O 1s, and Mn 2p. Mn 2p spectra (Fig. S2b) show two characteristic peaks at 653.1 eV and 641.6 eV which confirm the Mn 2p$_{3/2}$ and Mn 2p$_{1/2}$ orbits of the Mn$^{4+}$ oxidation state. These results confirmed the successful deposition of MnO$_2$ in NPs.

The cisplatin and MnO$_2$ contents of the Pt@mPDA/MnO$_2$/PDA NPs were quantified by inductively coupled plasma-atomic emission spectrometry (ICP-AES) to be ~96 mg/g and ~164 mg/g, respectively. Collectively, these results validate the successful construction of Pt@mPDA/MnO$_2$/PDA NPs.

TME-triggered NPs degradation and drug release in vitro

The degradation of NPs triggered by H$_2$O$_2$ and GSH was first studied by measuring the UV-Vis absorption of Pt@mPDA/MnO$_2$/PDA NPs in different media. As shown in Fig. S3, the NPs were stable under neutral pH (Fig. S3a), but showed a mild H$_2$O$_2$-sensitive degradation manner, as the absorption decreased over time in the PBS containing 1 mM H$_2$O$_2$ (Fig. S3b). In striking contrast, the absorption of the dispersed NPs decreased abruptly after supplementing with 2 mM GSH (Fig. S3c), and this decrease was further enhanced after increasing the GSH concentration to 5 mM (Fig. S3d). After 14 d, the absorption level and color of Pt@mPDA/MnO$_2$/PDA NPs under varied conditions were compared. Apparently, the NPs treated with 1 mM H$_2$O$_2$ and 5 mM GSH showed the greatest degradation degree (Fig. S3e and S3f). Correspondingly, the morphology of the NPs after 14 d by TEM (Fig. 1d) displayed H$_2$O$_2$- and GSH-sensitive degradation behavior. The former was ascribed to PDA accelerated degradation in the presence of H$_2$O$_2$ [42], while the latter was attributed to the redox reaction between the MnO$_2$ layer and GSH [15, 16]. These results suggest that the as-prepared mPDA/MnO$_2$/PDA NPs are promising biodegradable materials for TME-specific drug release and in vivo safe use.

The drug release behavior is presented in Fig. 1e. The Pt@mPDA/MnO$_2$/PDA NPs exhibited H$_2$O$_2$, GSH, and pH triple model-responsive drug release. In blank PBS (pH 7.4), the cisplatin was released slowly, with only ca. ~6.6% of the incorporated cisplatin released after 48 h, while a more rapid and an extensive drug release (ca. ~27.8% after 48 h) occurred after supplementing with 1 mM H$_2$O$_2$. In contrast, a greater extent of drug release (ca. ~88%) occurred after adding 5 mM GSH. These data support the degradation results. Owing to the mild acid pH-triggered degradation feature of MnO$_2$ [22], it was noted that the drug
release rate was further accelerated in acidic conditions (pH 5.5). Collectively, Pt@mPDA/MnO$_2$/PDA NPs exhibit tumor-specific drug release capability, thus can reduce off-target drug leakage.

**In vitro decomposition of H$_2$O$_2$ triggered by NPs and GSH-triggered MRI**

Solid tumor hypoxic microenvironment severely limits the RT efficacy, ascribing to the fact that O$_2$ promoted DNA damage and ROS generation induced by ionizing radiation [5, 6]. To address this hurdle, MnO$_2$ contained NPs were constructed as a catalyst to trigger the decomposition of H$_2$O$_2$ to O$_2$. As displayed in Fig. 1f, significant and sustained amounts of O$_2$ was generated after adding mPDA/MnO$_2$/PDA NPs ([MnO$_2$] = 2 μg/mL) into H$_2$O$_2$ contained PBS, while PBS with or without H$_2$O$_2$ in the absence of NPs maintained a stable O$_2$ concentration.

The GSH-activated T$_1$-MRI contrast performance of mPDA/MnO$_2$/PDA NPs was investigated with or without GSH treatment. As expected, the NPs showed much stronger T$_1$-weighted MR signal enhancement upon exposure to GSH compared to the NPs in the absence of GSH, with a 37.1-fold increase in r$_1$ value after treatment with GSH (Fig. 1g). Correspondingly, a concentration-dependent brightening effect of mPDA/MnO$_2$/PDA NPs upon treatment with GSH was observed (Fig. 1h), which can be attributed to the GSH-triggered MRI agent Mn$^{2+}$. These results confirm that the MnO$_2$-functionalized NPs can be served as promising candidates for tumor-specific imaging.

**Preparation and characterization of Pt@mPDA/MnO$_2$/PDA-Z$_{Her2}$ NPs**

The Z$_{Her2}$ affibody was expressed by the *E. coli* expression system and purified using Ni-NTA affinity chromatography. As shown in Fig. 2a, compared to the total protein from cells before induction (Fig. 2a, lane 1), a protein with a molecular weight of approximately 10 kDa, which was similar to its theoretical molecular weight of 8 kDa, was induced by IPTG (Fig. 2a, lane 2). Following cell disruption, SDS-PAGE analysis of the total protein in the soluble (Fig. 2a, lane 3) and insoluble (Fig. 2a, lane 4) fractions revealed that the recombinant protein was mainly expressed as a soluble form. After binding to Ni-NTA affinity resin, the recombinant protein in the flow-through fractions was reduced remarkably (Fig. 2a, lane 5), suggesting high purification efficiency. The purified Z$_{Her2}$ proteins were visualized as a single protein band on the SDS-PAGE gel, and the purity was calculated to be more than 95% by scanning densitometry of the electrophoretic bands by Image J (Fig. 2a, lane 6). Approximately 30–40 mg of protein was obtained from the cells in 1 L of culture.

The affinity of the dimeric form affibody for its target was usually higher than that of the monomer form [43]. Thus, the Z$_{Her2}$ affibody was designed to form a dimer upon the addition of a cysteine residue at the C-terminus. As expected, the molecular weight of Z$_{Her2}$ under natural conditions (Fig. 2b, in the absence of 2-ME) was approximately 2-fold that of Z$_{Her2}$ under reductive conditions (Fig. 2b, in the presence of 2-ME), indicating that Z$_{Her2}$ forms disulfide bond-containing dimers under natural conditions. These results demonstrated that the dimeric Z$_{Her2}$ affibody was successfully obtained.
A slight increase in molecular weight was observed after Z\textsubscript{Her2} was labelled with 6-Carboxyfluorescence (6-FAM) (Fig. 2c), which indicated successful labelling, and the labelling efficiency was approximately 100%. This allowing the visual evaluation of the binding capacity between FAM-Z\textsubscript{Her2} and Her2-positive cells.

Finally, the Z\textsubscript{Her2} affibody was coupled with Pt@mPDA/MnO\textsubscript{2}/PDA NPs via a Michael addition/Schiff base reaction by conjugating the amino group to the oxidized quinone form of catechol groups in weak alkaline conditions. The mean hydrodynamic size of NPs increased slightly from 185 nm to 201 nm after Z\textsubscript{Her2} affibody conjugation (Fig. S4). The elemental mapping images (Fig. 2d–2k) of mPDA/MnO\textsubscript{2}/PDA NPs displayed the existence of the homogeneous distribution of C, N, O, S, Mn, and Pt in NPs, thus further confirming the chemical composition and the successful loading of cisplatin, as well as coupling of Z\textsubscript{Her2}. In addition, the Z\textsubscript{Her2} content was approximately 0.8 mg/g by quantifying S (Z\textsubscript{Her2}-specific element) using ICP-AES.

**Her2-positive cell specific binding and cytotoxicity assay**

Flow cytometry (FCM) and confocal laser scanning microscopy (CLSM) results (Fig. S5a–c) showed concentration-dependent increase in fluorescence for human ovarian cancer cell line (SKOV-3 cells) after incubation with FITC-labelled anti-Her2 antibody, while negligible FITC fluorescence was probed in breast cancer cell line (MCF-7 cells). These results confirmed that SKOV-3 cells are Her2-positive expressed, while MCF-7 cells are Her2-negative expressed, which is consistent with the verified results as described previously [44]. The binding activity of FAM-Z\textsubscript{Her2} with Her2-negative MCF-7 cells and Her2-positive SKOV-3 cells was examined by CLSM. As shown in Fig. S6a, FAM-Z\textsubscript{Her2} specifically binds to SKOV-3 cells, while negligible FAM-Z\textsubscript{Her2} binding to MCF-7 cells was observed, indicating that these affibodies exhibit specific binding activity to Her2-positive cancer cells. This Her2-specific binding activity was further confirmed by FCM, as strong FAM fluorescence can be clearly probed in SKOV-3 cells; however, this specific binding was significantly reduced after pre-incubation with free Z\textsubscript{Her2} (Fig. S6b).

FCM analysis were done to evaluate the affinity of monomer and dimer of the affibody to Her2 receptor by measuring the FAM fluorescence intensity. As shown in Fig. S7a, the binding rates of the dimer Her2 affibody are 58.4% compared to 33.8% for monomer Her2 affibody at the same molar concentration. These results suggest that the affinity for Her2 receptor of the dimeric Her2 affibody was higher than that of the monomeric Her2 affibody.

To examine whether the Z\textsubscript{Her2} could enhance internalization of NPs into Her2 overexpressed cancer cells, the uptake of Cy5.5 labelled mPDA/MnO\textsubscript{2}/PDA (Cy5.5@mPDA/MnO\textsubscript{2}/PDA) and mPDA/MnO\textsubscript{2}/PDA-Z\textsubscript{Her2} (Cy5.5@mPDA/MnO\textsubscript{2}/PDA-Z\textsubscript{Her2}) NPs by SKOV-3 cells was studied by CLSM and quantified by FCM assay. As shown in Fig. 3a, minimal intracellular red fluorescence was observed in SKOV-3 cells after treatment with Cy5.5@mPDA/MnO\textsubscript{2}/PDA NPs. In striking contrast, strong Cy5.5 (red) fluorescence was visible in SKOV-3 cells after incubation with Cy5.5@mPDA/MnO\textsubscript{2}/PDA-Z\textsubscript{Her2} NPs. A blocking experiment
by pre-incubating SKOV-3 cells with free $Z_{\text{Her2}}$ (20 μg/mL) was performed to further explore the mechanism of endocytosis. CLSM images showed attenuated fluorescence signal inside SKOV-3 cells, indicating that the reduced endocytosis, presumably ascribed to occupied Her2 receptors, failed to bind NPs-conjugated $Z_{\text{Her2}}$. Correspondingly, the FCM data (Fig. 3b) showed consistent results, in which the uptake of cellular NPs significantly improved after conjugating $Z_{\text{Her2}}$ compared to that of Cy5.5@mPDA/MnO$_2$/PDA NPs; however, the cellular uptake was significantly reduced after blocking the binding sites of $Z_{\text{Her2}}$. Collectively, these results confirm that the $Z_{\text{Her2}}$ affibody can enhance the internalization of NPs into Her2-positive cancer cells, which attributing to the specific affinity effect between $Z_{\text{Her2}}$ affibody and the Her2 receptor.

The cytocompatibility of drug-free mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs with human umbilical vein endothelial cells (HUVECs) was first examined by MTT assay. As shown in Fig. S7b, negligible toxicity to non-cancerous cells was observed, even when the concentration of mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs reached 250 μg/mL (with above 90% viability), thus demonstrating the good cytocompatibility of the carrier materials.

All cisplatin-containing formulations exhibited dose-dependent cytotoxicity to SKOV-3 cells (Fig. 3c). The half maximal inhibitory concentrations ($IC_{50}$) of free cisplatin, Pt@mPDA/MnO$_2$/PDA NPs, and Pt@mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs to SKOV-3 cells were calculated to be 9.79 ± 0.8 μg/mL, 3.18 ± 0.3 μg/mL, and 2.56 ± 0.1 μg/mL, respectively. In contrast to that of free cisplatin, the Pt@mPDA/MnO$_2$/PDA NPs exhibited more potent toxicity to cells over the entire cisplatin dose range (0.6–48 μg/mL), which can be ascribed to more efficient cellular uptake of NPs. More encouragingly, the conjugation of $Z_{\text{Her2}}$ to Pt@mPDA/MnO$_2$/PDA NPs further improved cytotoxicity. This can be explained by efficient internalization mediated by the targeted affinity of $Z_{\text{Her2}}$ on the NPs to Her2 receptors of SKOV-3 cells.

Calcein-AM/PI double staining was performed to evaluate the degree of apoptosis of the cells. The optimal degree of cell apoptosis was observed upon treatment with Pt@mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs (Fig. 3d), which is consistent with the MTT results.

O$_2$, as the ROS-generating resource induced by X-Ray, we thus evaluated the effect of MnO$_2$-contained NPs on the intracellular oxidative stress levels. As shown in Fig. 3e, compared to X-Ray alone, the treatment of MnO$_2$-free mPDA/PDA-$Z_{\text{Her2}}$ NPs combined with X-Ray did not increase intracellular ROS level, as low DCF fluorescence was observed for X-Ray with or without mPDA/PDA-$Z_{\text{Her2}}$ NPs treated cells. The intracellular oxidative stress levels of SKOV-3 cells improved after treating with mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs alone, this can be ascribed to ability to produce HO• (one ROS species) of MnO$_2$ in tumor cells [15, 16]. In stark contrast, for the mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs and X-Ray combined, the intracellular ROS level was largely increased compared with that of MnO$_2$-free mPDA/PDA-$Z_{\text{Her2}}$ NPs + X-Ray. This finding indicates that the treatment with MnO$_2$-contained NPs can facilitate ROS generation induced by ionizing radiation, which exhibiting great potential for radiosensitivity.

Tumor targeting profiles in vivo
The presence of $Z_{\text{Her2}}$ in NPs inspired us to expect the Her2-overexpressed tumor targeting capacity of Pt@mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs. The TME-triggered MRI ability of NPs was first investigated. As shown in Fig. 4a and 4b, the $T_1$-weighted MRI signal in the tumor increased gradually over time, while negligible MRI signal enhancement was displayed in the muscle area. This can be attributed to the reduction of the MnO$_2$ layer to the MRI agent Mn$^{2+}$ by TME-overexpressed GSH [16, 45], which makes the MnO$_2$-contained NPs particularly attractive for tumor-specific imaging. Then, the tumor accumulation of Pt@mPDA/MnO$_2$/PDA and Pt@mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs in SKOV-3 tumor-bearing mice at different time points after intravenous injection was evaluated by tracking the MRI signal. As depicted in Fig. 4c, an MRI signal was observed at the tumor site 1 h post-injection of Pt@mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs, and the intensity increased gradually over time, indicating its efficient accumulation in the tumor. In contrast, reduced tumor accumulation of Pt@mPDA/MnO$_2$/PDA NPs was observed. Obviously, the signals from Pt@mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs were significantly stronger than those from Pt@mPDA/MnO$_2$/PDA NPs ($p < 0.05$) at 6 h (Fig. 4d). The biodistribution result of Pt@mPDA/MnO$_2$/PDA and Pt@mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs in SKOV-3 tumor by quantifying Pt content using ICP-AES at 24 h post-injection was presented in Fig. S8. The Pt content in tumors of mice treated with Pt@mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs was 1.5-fold higher than the Pt content in mice that received Pt@mPDA/MnO$_2$/PDA NPs ($p < 0.05$), supporting the tumor-targeted ability rendered by $Z_{\text{Her2}}$ abody.

Immunofluorescence and bio-TEM assays were performed to further evaluate the tumor-targeting ability of Cy5.5@mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs on the histological level. Immunofluorescence staining images showed that Cy5.5 fluorescence (representing the Cy5.5@mPDA/MnO$_2$/PDA NPs) was mainly restricted to tumor peripheral tissues (Fig. 5a and 5c(i)), suggesting poor penetration of NPs. Strong encouragingly, under the guidance of $Z_{\text{Her2}}$, the NPs overcame biological barriers and penetrated deeply into the tumor tissues. As shown in Fig. 5b and 5c(ii), strong red fluorescence intensity can be seen throughout the whole tumor tissue and co-localized with green fluorescence (representing Her2). These results suggest efficient tumor homing and penetration of Cy5.5@mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs attributing to the efficient internalization and penetrability of $Z_{\text{Her2}}$.

This effect was also confirmed by bio-TEM images (Fig. 6) of tumor tissues. Greater numbers of Cy5.5@mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs were present within the cytoplasm, while only a few Cy5.5@mPDA/MnO$_2$/PDA NPs can be observed in tumor tissues. This superior tumor penetration and targeting capabilities are crucial for enhancing therapeutic efficacy. Collectively, these findings suggest that with the assistance of $Z_{\text{Her2}}$ modification, the TME-triggered off-to-on diagnostic agent makes Pt@mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$ NPs particularly attractive for MRI-guiding tumor-targeted treatment.

**In vivo chemo-sensitized radiotherapy**

Encouraged by the tumor-targeted homing and penetration effect of Pt@mPDA/MnO$_2$/PDA-$Z_{\text{Her2}}$, we then assessed the *in vivo* chemotherapeutic activity in SKOV-3 tumor-bearing mice. As shown in Fig. 7a, for
the mice treated with PBS, the volume of tumors expanded sharply during the treatment period. The mice that were administered free cisplatin did not exhibit appreciable tumor suppression, probably due to the insufficient accumulation of cisplatin in tumors. In a sharp contrast, for the mice that were administered Pt@mPDA/MnO$_2$/PDA NPs, tumor progression was significantly inhibited as a result of sufficient tumor retention of cisplatin-containing NPs. Furthermore, with the guidance of Z$_{\text{Her2}}$ affibody, Pt@mPDA/MnO$_2$/PDA-Z$_{\text{Her2}}$ NPs achieved the most potent inhibition of tumor growth, and by the end of the treatment, these extracted tumors displayed the smallest volume and mass (Fig. 7b and 7c), persuasively demonstrating the targeted antitumor activity. Body weight was monitored every day, and no obvious body weight difference was observed among all the treated groups (Fig. 7d).

A TUNEL assay was conducted to further study the apoptosis level of tumor tissues. The largest number of green-colored cells (indicating the highest levels of apoptosis) were observed for Pt@mPDA/MnO$_2$/PDA-Z$_{\text{Her2}}$ NPs-treated tumor tissues (Fig. 7e), indicating the most potent antitumor effect.

Inspired by the remarkable tumor growth inhibition effect of Pt@mPDA/MnO$_2$/PDA-Z$_{\text{Her2}}$ NPs, we next assessed the combined chemo-radiation therapeutic efficiency, especially the enhanced RT effect, by adopting MnO$_2$ as a radiosensitizer for hypoxic tumors. The treatment schedule for chemo-radiotherapy is illustrated in Fig. 8a. RT alone did not have any remarkable antitumor effect, as a result of the RT resistance caused by hypoxic TME. A sharply growth in tumor size (Fig. 8b–d) was seen during the treatment period. In the combined treatment group of Pt@mPDA/MnO$_2$/PDA-Z$_{\text{Her2}}$ NPs with X-Ray radiation, the tumors showed the slowest growth rate and smallest size at the end of treatment, indicating the combined effect of chemotherapy and radiotherapy. Particularly, the mice receiving Pt@mPDA/MnO$_2$/PDA-Z$_{\text{Her2}}$ NPs and X-Ray radiation exhibited the most profound inhibition of tumor growth, which was significantly stronger than that of the MnO$_2$-free Pt@mPDA/PDA-Z$_{\text{Her2}}$ NPs + X-ray treatment ($p < 0.001$), indicating sensitized RT effect induced by MnO$_2$. No obvious body weight changes were observed with any of the treatments (Fig. 8e).

To better understand the action and reveal the RT sensitivity mechanism, the HIF-1$\alpha$ expression level of tumor tissues extracted from different groups was determined by immunofluorescence assay. As shown in Fig. 8f, compared with that in all MnO$_2$-free formulation treatment groups, the tumor tissues from the mice treated with Pt@mPDA/MnO$_2$/PDA NPs showed remarkably reduced red fluorescence signals (HIF-1$\alpha$), indicating that the relieved hypoxic TME, which is attributed to the decomposition of endogenous H$_2$O$_2$ to O$_2$ by MnO$_2$. Furthermore, with the targeted guiding of Z$_{\text{Her2}}$ affibody, HIF-1$\alpha$ expression was further reduced by Pt@mPDA/MnO$_2$/PDA-Z$_{\text{Her2}}$ NPs, supporting the tumor-targeted ability rendered by Z$_{\text{Her2}}$ affibody again and thus triggered the sensitized RT effect.

Safety evaluation
The off-target toxicity is always a prerequisite when employing materials for *in vivo* use or clinical translation, so the biocompatibility and biosafety of Pt@mPDA/MnO₂/PDA-ZHER₂ NPs were systematically evaluated by histology and serum biochemistry assays. Interestingly, H&E images of major organs from all treatment mice showed no obvious tissue damage, compared with that of the PBS group (Fig. S9), suggesting that the drug, materials, or both did not cause any significant systemic toxicity. Similarly, all serum biomarkers (ALT, AST, UREA, CREA, and UA) were not different from those of the control group (Fig. S10). This probably can be attributed to the relatively low side effect of clinical cisplatin and biomimetic PDA as well as the short treatment period. Therefore, this biocompatible nanotheranostic agent exhibits promising potential for clinical translation.

Her2, overexpressed in a variety of human cancers, is closely related to cell proliferation, differentiation, adhesion, migration, and anti-apoptosis as well as poor prognosis and rapid recurrence of tumor, and thus has received great attention in antitumor studies over the past two decades [44]. For example, antibody-drug conjugates (ADCs, such as FDA approved trastuzumab, pertuzumab, and T-DM1), which integrate Her2-specific targeting antibodies with high cytotoxicity of small-molecule chemotherapeutic agents, possess the ability to selectively deliver highly potent cytotoxic drugs to tumor sites, and thus have become a powerful cancer-targeted treatment approach. However, these ADCs generally face problems with no TME-response and absence of diagnosis, as well as expensive cost, which limits its wide usage. Herein, a Her2 targeting ZHER₂ affibody was yielded by a genetic engineering approach, and succeeded in coupling with Pt@mPDA/MnO₂/PDA NPs is reported in this study. Compared to the widely used intact antibody, this engineered affibody has advantages of higher specific affinity, stronger tissue penetrability, high yield at a low price, and low immunogenicity [44, 46]. Thus, it can be employed to guide nanoagents for enhanced therapeutic efficacy. In addition, in marked contrast to monofunctional antibody-drug conjugates, we integrated ZHER₂ affibody with nanoagent featuring specific targeting, RT sensitization, and diagnosis, providing a new idea for clinical translation in tumor theranostics.

**Conclusion**

In this study, we report a Her2-targeted cisplatin-loaded mPDA/MnO₂/PDA NPs for MR imaging and enhanced chemo-radiotherapy of hypoxic tumors. These NPs are biodegradable under a simulated tumor microenvironment, resulting in accelerated cisplatin release as well as O₂ production by triggering decomposition of H₂O₂. Cell uptake experiments demonstrated that ZHER₂ endows NPs targeted to bind to Her2, and thus achieved enhanced internalization of NPs into Her2-positive SKOV-3 cells. *In vivo* MRI studies revealed an obvious T₁ contrast enhancement at the tumor site. Immunofluorescence assay showed that the presence of MnO₂ significantly reduced the expression of HIF-1α, and that the Cy5.5 fluorescently labelled carrier has a high affinity to Her2. Chemotherapy results verified that Pt@mPDA/MnO₂/PDA-ZHER₂ NPs have a strong targeted antitumor efficacy. Under X-Ray irradiation, the Pt@mPDA/MnO₂/PDA-ZHER₂ NPs can relieve tumor hypoxia, exhibiting the highest tumor growth inhibition effect. Hence, this multifunctional nanoplatform shows promising potential for clinical translation in tumor theranostics.
Abbreviations

RT: Radiotherapy; ROS: Reactive oxygen species; TME: Tumor microenvironment; HIF-1α: Hypoxia-inducible factor 1α; MRI: Magnetic resonance imaging; Her2: Human epidermal growth factor receptor 2; Pt: Cisplatin; mPDA: Mesoporous polydopamine.

Declarations

Ethics approval and consent to participate

All animal experiments were carried out with full authorization approved by the ethical committee for animal care of Shandong Cancer Hospital and Institute Shandong, First Medical University and Shandong Academy of Medical Sciences (Approval No. SDTHEC2020004083).

Consent for publication

All authors agree to be published.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

HJW, QF and LMZ designed the experiment, analysed the data and drafted the manuscript. HJW prepared and characterized the Pt@mPDA/MnO$_2$/PDA-Z$_{\text{Her2}}$ NPs, FFW and DLJ prepared Z$_{\text{Her2}}$. HJW and QF performed the animal experiments. DDY, XLY, FJY, WNS and NL assisted with the in vitro and in vivo experiments. All authors read and approved the final manuscript.

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