Intracellular marriage of bicarbonate and Mn ions as “immune ion reactors” to regulate redox homeostasis and enhanced antitumor immune responses

Yushuo Feng, Yaqing Liu, Xiaoqian Ma, Lihua Xu, Dandan Ding, Lei Chen, Zongzhang Wang, Ruixue Qin, Wenjing Sun and Hongmin Chen*

Abstract

Background: Different from Fe ions in Fenton reaction, Mn ions can function both as catalyst for chemodynamic therapy and immune adjuvant for antitumor immune responses. In Mn-mediated Fenton-like reaction, bicarbonate ($\text{HCO}_3^-$), as the most important component to amplify therapeutic effects, must be present, however, intracellular $\text{HCO}_3^-$ is strictly limited because of the tight control by live cells.

Results: Herein, Stimuli-responsive manganese carbonate-indocyanine green complexes (MnCO$_3$-ICG) were designed for intracellular marriage of bicarbonate and Mn ions as “immune ion reactors” to regulate intracellular redox homeostasis and antitumor immune responses. Under the tumor acidic environment, the biodegradable complex can release “ion reactors” of Mn$^{2+}$ and $\text{HCO}_3^-$, and ICG in the cytoplasm. The suddenly increased $\text{HCO}_3^-$ in situ inside the cells regulate intracellular pH, and accelerate the generation of hydroxyl radicals for the oxidative stress damage of tumors cells because $\text{HCO}_3^-$ play a critical role to catalyze Mn-mediated Fenton-like reaction. Investigations in vitro and in vivo prove that the both CDT and phototherapy combined with Mn$^{2+}$-enhanced immunotherapy effectively suppress tumor growth and realize complete tumor elimination.

Conclusions: The combination therapy strategy with the help of novel immune adjuvants would produce an enhanced immune response, and be used for the treatment of deep tumors in situ.

Keywords: Self-supplying intracellular ions, Redox homeostasis, Immune activator, Manganese immunotherapy, Orthotopic liver cancer
which is the most important component to amplify therapeutic effects [23]. The development of stimuli-responsive nanomedicine is appealing to achieve effective Mn-mediated Fenton-like cancer treatment. Thus, as a typical pH-dissociable Mn-based biominal, MnCO$_3$ formulations have excellent potential to achieve these purposes because they have similar biodegradability and biocompatibility as CaCO$_3$ [24–27].

The enhancement of tumor therapy relies not only on the development of biocompatible nanoformulations but also on the discovery and implementation of clinically capable therapeutic mechanisms. Over the past decades, manganese-based nanostructures have drawn much attention in biomedical applications. For instance, Mn$^{2+}$ salts or complexes have been used in clinical MR imaging [28–31] and can induce tumor cell apoptosis [15–17, 22, 27, 32]. Recent studies have revealed that Mn can lead to excellent tumor-ablation therapeutic effect, although the mechanism and role of Mn have not been completely explored. More evidences have indicated that there are two explanations for the enhanced therapy efficacy:

(1) a Mn-mediated Fenton-like reaction is strongly enhanced by HCO$_3^-$.[33–37] Carbonate concentration and pH play essential roles in the formation of catalytically active species that maintains the high oxidation reactivity of the oxidizing intermediate [38, 39]. Mammalian tissues are bathed in a milieu that typically contains HCO$_3^-$ at a concentration of about 25 mM, and cells have developed a mechanism to maintain an intracellular concentration of about 12 mM [40]. CO$_2$ is the end product of mitochondrial energy production and can ultimately be converted into HCO$_3^-$ in the cytoplasm [40–42]. In an acidic environment, carbonate reacts with the acid to quickly generate CO$_2$ bubbles [43, 44], which regulate the local intracellular pH and generate HCO$_3^-$ in situ; this boosts the Mn-mediated Fenton-like reaction. Governing pH regulation using bicarbonate has been carefully studied, and CO$_2$/HCO$_3^-$ is important for maintaining uniform alkaline pH in small, non-vascularized tumors; it is an important target for cancer progression [45, 46]. The Gillies group comprehensively showed that biocarbonate plays an indispensable role in cancer management, including inhibiting spontaneous metastases and improving antitumor responses to immunotherapy [47]. A common formula, sodium bicarbonate, known as baking soda, is widely used in the clinic as an acidid for treating gastric hyperacidity [48]. The Hu group completed a clinical trial into targeting intratumoral lactic acidosis-transarterial chemoembolization for large hepatocellular carcinoma (HCC) (TILA-TACE) (Clinical trial number: ChiCTR-IOR-14005319) [49]. Following the TILA-TACE procedure (widely employed for the local control of HCC), sodium bicarbonate plus cytotoxic drugs resulted in a high local control rate [50]. The authors stated that the possible reason is related to acidosis in the tumor microenvironment. Although this is a small-scale pilot study with limitations on cancer types and delivery method (sodium bicarbonate highly hydrolyzed), the results still offered the potential to exploit tumor acidosis from “the passenger” to “the driver’s seat” [40, 48, 51, 52]. This was done in combination with other anticancer therapies in treating various malignancies.

(2) Mn$^{2+}$ strongly promotes immune responses. Immunotherapy has revolutionized clinical cancer treatment and reached conspicuous successes. Recent studies suggest that Mn$^{2+}$ would strongly promote immune responses through proliferating cytotoxic T lymphocyte and promoting dendritic cell maturation [17, 53–55]. These reports imply that the development of Mn-based complexes combined with traditional treatment of cancer have great potential to improve the antitumor effect [15, 56]. Currently, only six adjuvants have been approved for clinical use, including Alum, MF59, AS04, AS03, AS01 and CpG 1018 [57]. Thus, developing novel adjuvants could induce a high magnitude of immunity response. Nanosized sustained release system can enhance the magnitude, quality, and persistence of immunity responses.

Herein, on the basis of the insights from the preliminary results, we designed a MnCO$_3$-based nanocomplexes with amplification of tumor oxidative stress, regulation of intracellular redox homeostasis, and enhancement of immune response (Scheme 1). In our design, indocyanine green (ICG), an FDA-approved clinically used fluorescent agent for diagnostics and imaging-guided surgery, first chelates with Mn ions and co-condenses with CO$_2$ to produce Mn carbonate-indocyanine green complexes (MnCO$_3$-ICG) [58, 59]. The complexation of ICG and Mn ions facilitates efficient loading of imaging agents and also minimizes the susceptibility of Mn ions to intrahost oxidative stress before their intracellular release. Meanwhile, the MnCO$_3$ is sacrificed via hydrolysis and releasing Mn$^{2+}$ ions, ICG, and CO$_3^{2-}$ into the cytosol. Compared with the other Mn-induced CDT strategies by solely increasing the intracellular Mn accumulation, the in situ self-supplied HCO$_3^-$ pool serves as “immune ion reactors” to increase the ROS generation in the tumor cells and boost DC maturation and increase CD8$^+$ T cells. The released ICG molecules could penetrate into deep tumors and further increase the therapeutic effects by imaging-guided phototherapy. Considering the negligible systemic biological toxicity of Mn$^{2+}$, CO$_3^{2-}$, and ICG, we believe the released components from the pH-triggered decomposition of MnCO$_3$-ICG formulation is anticipated to act synergistically to amplify the oxidative stress, regulate intracellular redox homeostasis
and activate the immune response, leading to enhanced tumor cell death in an on-demand manner.

**Materials and methods**

**Synthesis of manganese carbonate-indocyanine green complexes (MnCO$_3$-ICG)**

MnCO$_3$-ICG complexes were synthesized by adopting the gas diffusion process. Briefly, MnCl$_2$·4H$_2$O (10 mg/mL, 5 mL) and different concentrations of ICG were mixed with 50 mL ethanol. After stirred 3 h, the mixture (Mn-ICG) was placed in a sealed container with NH$_4$HCO$_3$ and kept at room temperature. After 0.5, 1, 2 or 4 h, the green MnCO$_3$-ICG complexes were collected and purified by repeated centrifugation at 5000 rpm. The PAH (5 mg/mL) solution was added into 50 mL of ethanol containing different concentrations of MnCO$_3$-ICG complexes to obtain excellent aqueous solubility. The mixture was then stirred for 1 h at room temperature. Finally, the obtained MnCO$_3$-ICG complexes were collected and purified by centrifugation at 10,000 rpm for 10 min.

**The hydroxyl radicals (-OH) generation by Mn$_{2+}$-mediated Fenton-like reaction**

MnCO$_3$-ICG ([Mn]: 50 μg/mL) were incubated with different buffer solution (pH 5.8; pH 7.4 with 20 mM NaHCO$_3$; pH 7.4 with 20 mM NaHCO$_3$) for 30 min. After centrifugation, the MB (10 μg/mL) and H$_2$O$_2$ (1 mM) were added to the supernatant. The absorbance of MB was measured after shaking at 37 °C for 30 min.
Enhanced-chemodynamic activity of MnCO₃-ICG complexes

MnCO₃-ICG ([Mn]: 50 μg/mL) were incubated with different different concentrations of NaHCO₃ in the acidic environment for 30 min. After centrifugation, the MB (10 μg/mL) and H₂O₂ (1 mM) were added to the supernatant. The absorbance of MB was measured after shaking at 37 °C for 30 min.

MnCO₃-ICG and Mn-ICG ([Mn]: 50 μg/mL) were incubated with different buffer solution (pH 7.4; pH 5.8; pH 7.4 with 1 mM H₂O₂; pH 5.8 with 1 mM H₂O₂) for 30 min. After centrifugation, the MB (10 μg/mL) and H₂O₂ (1 mM) were added to the supernatant. The absorbance of MB was measured after shaking at 37 °C for 30 min.

MnCO₃-ICG ([Mn]: 50 μg/mL) were incubated in the acidic environment (pH 5.8) for different time. After centrifugation, the MB (10 μg/mL) and H₂O₂ (1 mM) were added to the supernatant. The absorbance of MB was measured after shaking at 37 °C for 30 min.

Cell uptake

To evaluate the cell uptake efficiency, 4T1 cells were seeded in six-well plates at a density 10⁵ cells for 24 h. After that, the cells incubated with MnCO₃-ICG ([Mn]: 5 μg/mL) in the dark for different time (2, 4, 8, 12, and 24 h). After washing with PBS for three times, the cells were stained with hoechst33342 (5 μg/mL) and imaged by a laser scanning confocal fluorescence microscope.

For quantitative analysis the cell uptake efficiency, 4T1 cells were seeded in six-well plates at a density 10⁵ cells for 24 h. After that, the cells incubated with MnCO₃-ICG ([Mn]: 5 μg/mL) in the dark for different time (2, 4, 8, 12, and 24 h). After washing with PBS for three times, the cells were digested and analyzed by flow cytometer (ACEA Biosciences).

4T1 cells were also seeded in observation dish at a density 10⁵ cells for 24 h, and incubated with MnCO₃-ICG ([Mn]: 5 μg/mL) in the dark for 24 h. After washing with PBS for three times, the cells were stained with hoechst33342 (5 μg/mL) and lyso-tracker (2 μM). The stained cells were imaged by a laser scanning confocal fluorescence microscope.

In vitro photothermal and chemodynamic therapy effect

To evaluate the chemodynamic therapy effect, 4T1 cells, U87MG cells and Hep G2 cells were seeded in 96-well plates (10⁴ per well) and incubated with different concentrations of MnCO₃-ICG for 24 h or 48 h. Cells viability were determined by the MTT assay. The photothermal therapy effect was detected by exposing cells to NIR laser (808 nm, 0.5 W/cm²) for 5 min. Cells viability were also determined by the MTT assay.

To visually observe the living and dead cells, 4T1 cells were seeded in six-well plates at a density 10⁶ cells for 24 h. Then, the cells were co-incubated with MnCO₃-ICG ([Mn]: 5 and 20 μg/mL) for 24 h. The photothermal therapy group were exposed to NIR laser (808 nm, 0.5 W/cm²) for 5 min. After that, the cells were continue incubated for another 24 h, and staining with AM/PI dual-staining kit. After washing with PBS for three times, the cells were imaged by an inverted fluorescence microscope.

The therapy effect of MnCO₃-ICG under the MCSs

4T1 cells (3000 cells per well) were seeded into 96-wells plate containing 1% agarose to form MCSs. The MnCO₃-ICG ([Mn]: 20 or 50 μg/mL) were added to the dishes of the MCSs for 24 h. Then, the photothermal therapy group were exposed to NIR laser (808 nm, 0.5 W/cm²) for 5 min. The MCSs were continue incubated for another 24 h, and staining with AM/PI dual-staining kit. After washing with PBS for three times, the MCSs were imaged by a laser scanning confocal fluorescence microscope.

In vivo photothermal and chemodynamic therapy effect

When the tumor reached 60 mm³ in average volume, the mice were randomly divide d into 6 groups: (Group 1) PBS (four doses); (Group 2) MnCO₃-ICG ([Mn]:2 mg/kg, four doses, termed as CDT-1); (Group 3) 2 × MnCO₃-ICG ([Mn]:4 mg/kg, four doses, termed as CDT-2); (Group 4) 4 × MnCO₃-ICG ([Mn]:8 mg/kg, four doses, termed as CDT-3); (Group 5) MnCO₃-ICG ([Mn]:2 mg/kg+L (0.5 W/cm²), one dose, termed as PTT); and (Group 6) MnCO₃-ICG ([Mn]:2 mg/kg+L (0.5 W/cm²), three doses, termed as PTT+CDT). The laser (808 nm, 0.5 W/cm², 5 min) was applied to tumor at 4 h post-injection of MnCO₃-ICG. The body weight and tumor size of the mice were recorded for the next 14 days. The relative tumor volume of each mouse was acquired by dividing by the initial tumor volume Tumor volumes and body weight were also recorded. After 14 days, the mice were euthanized and the collected tumor and major organ were kept for H&E staining.

Cytokine detection

IL-6 (Elabscience), TNF-α (Elabscience) and IL-1α (Elabscience) in mouse serum samples after different treatment were analyzed with ELISA kits according to the vendors’ protocols.

Ex vivo analysis of different groups of T cells and dendritic cells (DCs)

To analyze immune cells by flow cytometry, spleens of mice after various treatments were collected and
stained according to the manufacturer’s protocols. In brief, to analyze memory T cells, cells from tumors spleens were stained with antibodies against CD8a-APC (BioLegend, catalog no. 100712) and CD3-PE (BioLegend, catalog no. 100206). To analyze the maturation of DCs, cells from tumors spleens were stained with antibodies against CD80-APC (BioLegend, catalog no. 104714), CD86-PE (BioLegend, catalog no. 159204), and CD11c-FITC (BioLegend, catalog no. 117306).

**Results and Discussion**

**Preparation and characterization of MnCO$_3$-ICG complexes**

Non-spherical MnCO$_3$-ICG complexes were prepared through a gas diffusion procedure, which are monodisperse with an average diameter of 72 nm (Fig. 1A). The morphology and structure of MnCO$_3$-ICG would not change by increasing the feeding ratio of ICG and MnCl$_2$·4H$_2$O (W/W) (Additional file 1: Fig. S1). Interestingly, spherical Mn-ICG complexes were formed in the absence of the natural decomposition of NH$_4$HCO$_3$ in an enclosed chamber (Additional file 1: Fig. S1). Elemental mapping indicated that Mn, C, O, N, and S were homogeneously distributed in the structure of MnCO$_3$-ICG (Fig. 1B). The absorbance spectra of MnCO$_3$-ICG showed typical peaks indicating the existence of ICG in the formed MnCO$_3$-ICG (Additional file 1: Fig. S2A). With uniform and well-defined...
structures, the MnCO₃-ICG prepared at a mass feeding ratio of ICG:MnCl₂·4H₂O = 2:50 and reaction time of 2 h in the NH₄HCO₃ environment were selected for further experiment (Additional file 1: Fig. S2B). The Mn and ICG contents were determined to be 20.0% and 18.2%, respectively. The stability of complexes played an important role for further bioapplications. Poly(allylamine hydrochloride) (PAH) were modified to increase the stability in physiological environment (Fig. S3A). MnCO₃-ICG exhibited excellent stability in water, saline solution, fetal bovine serum (FBS), and cell culture medium (DMEM) (Additional file 1: Fig. S3B) and there were no size changes after incubation for 24 h (Additional file 1: Fig. S3C). According to the dispersion and stability of the material, we conduct follow-up evaluation at rate weight of 1:5.

The stimulated response of MnCO₃-ICG

The realization of a Mn-mediated Fenton-like catalyzed reaction of H₂O₂ essentially relies on pH conditions, i.e., the concentration of HCO₃⁻. Therefore, we first studied the release kinetics of MnCO₃-ICG in biomimetic environment by incubating the material in various pH buffer solutions. TEM data showed that the structures of MnCO₃-ICG were stable at pH 7.4, but dissociated and re-assembled under mildly acidic condition (Fig. 1C). In the X-ray diffraction (XRD) (Additional file 1: Fig. S4A) and X-ray photoelectron spectroscopy (XPS) (Additional file 1: Fig. S4B–D) analysis of MnCO₃-ICG and the re-assembled nanoformulation, the structure turned from MnCO₃ to Mn₂O₃, and the valence states of Mn turned from Mn(II) to Mn(IV), after being dissociated and re-assembled. The decomposition profiles of MnCO₃-ICG were measured using MR and US phantom investigations. Under mildly acidic condition, the Mn²⁺ ion release increased gradually and then increased sharply with existing H₂O₂ and GSH, indicating the time-dependent T₁ intensity change (Fig. 1D, E). Notably, GSH would accelerate the decomposition of MnCO₃-ICG because it can be degraded by •OH (Additional file 1: Fig. S6C). To further test the importance of the generation of •OH, Figure 1H showed a drop in MB absorbance when MB was incubated with MnCO₃-ICG at pH 5.8 (reduced by 10.6%, green curve); a rapid degradation of MB was found by H₂O₂ plus HCO₃⁻ at pH 7.4 (reduced by 28.3%, blue curve) and pH 5.8 (reduced by 99.9%, red curve). We also examined the •OH production capacity of MnCO₃-ICG by electron paramagnetic resonance (EPR) spectroscopy. In comparison with H₂O₂ alone, obvious characteristic peaks (1:2:2:1) were recognized in Mn²⁺/HCO₃⁻-mediated Fenton-like reaction group (Fig. 1I). Thus, these results suggested that HCO₃⁻ released from degraded MnCO₃-ICG played a beneficial role. Furthermore, the absorbance of MB dropped sharply by increasing pH 5.8 in PBS with the same concentration of HCO₃⁻ (red curve in Fig. 1H).

To further confirm that the in situ self-supplying HCO₃⁻-pool accelerates the Mn-mediated Fenton-like reaction, Mn-ICG complexes were also incubated with MB plus H₂O₂ at pH 7.4 and 5.8. A significant decrease in MB absorbance was observed in MnCO₃-ICG (Additional file 1: Fig. S6B). Remarkably, the absorbance of MB degraded by 43.1% after MnCO₃-ICG incubated for 8 h at pH 5.8 without extra HCO₃⁻ (Additional file 1: Fig. S6C). To further test the importance of the generation of HCO₃⁻, we also removed the HCO₃⁻ (CO₂) by vacuuming the reactor for 10 min. It was clear that the degradation of MB would decline (Additional file 1: Fig. S7A, B). These results demonstrate that the Mn²⁺ and HCO₃⁻ were generated from MnCO₃-ICG, and the in situ self-supplying HCO₃⁻-pool accelerated Mn-mediated Fenton-like reaction.

Small organic molecules, including drugs (DOX, EGCG) and photosensitizers (TCPP, ICG) have been described to be able to coordinate with metal ions in recent discoveries [59, 62, 64–67]. Our MnCO₃-based nanocomplexes use the clinically approved fluorescent method was also adopted to study the generation of CO₂.

In vitro chemodynamic and photothermal therapy performance

After confirming the generation of Mn²⁺ and HCO₃⁻ under mildly acidic condition, we next evaluated the activity of the Mn-mediated Fenton-like reaction. Several recent discoveries have showed that HCO₃⁻-dependent peroxidation and H₂O₂ decomposition catalyzed by Mn²⁺ efficiently amplify intracellular oxidative stress via increasing •OH generation and reducing GSH biosynthesis [32, 63]. Methylene blue (MB) was chosen here as the probe because it can be degraded by •OH. Figure 1H showed a drop in MB absorbance when MB was incubated with MnCO₃-ICG at pH 5.8 (reduced by 10.6%, green curve); a rapid degradation of MB was found by H₂O₂ plus HCO₃⁻ at pH 7.4 (reduced by 28.3%, blue curve) and pH 5.8 (reduced by 99.9%, red curve). We also examined the •OH production capacity of MnCO₃-ICG by electron paramagnetic resonance (EPR) spectroscopy. In comparison with H₂O₂ alone, obvious characteristic peaks (1:2:2:1) were recognized in Mn²⁺/HCO₃⁻-mediated Fenton-like reaction group (Fig. 1I). Thus, these results suggested that HCO₃⁻ released from degraded MnCO₃-ICG played a beneficial role. Furthermore, the absorbance of MB dropped sharply by increasing pH 5.8 in PBS with the same concentration of HCO₃⁻ (red curve in Fig. 1H).
dye ICG; it has value in both photodynamic therapy and photothermal therapy [68, 69]. The MnCO$_3$-ICG showed strong absorption at 808 nm indicating that ICG was successfully incorporated into the ICG structure (Additional file 1: Fig. S8). MnCO$_3$-ICG solutions ([ICG]: 18.2, 36.4 and 72.8 μg/mL) were exposed to an 808 nm laser (0.5 W/cm$^2$), and the maximum temperature can reach up to 53.3 °C within 5 min (Fig. 1J). No obvious photobleaching of MnCO$_3$-ICG was observed after exposure to 808 nm laser (0.5 W/cm$^2$) for three laser on/off cycles compared with Mn-ICG and free ICG (red curve in Fig. 1K). Interestingly, Mn-ICG showed obvious photobleaching than that of free ICG (blue curve in Fig. 1K).

**In vitro evaluation of the therapeutic effect of MnCO$_3$-ICG**

We next studied the cellular internalization profile of MnCO$_3$-ICG in murine 4T1 breast cancer cells by using confocal microscopy. By recording the fluorescence of ICG, clear red fluorescence signals were observed in the

---

**Fig. 2** The Efficiently Induce Tumor Cell Death by MnCO$_3$-ICG. A Cell uptake of 4T1 cells incubated with MnCO$_3$-ICG at different time (scale bar, 50 μm). B Flow cytometric and quantitative analyses of internalization of MnCO$_3$-ICG at different time. C CLSM evaluation on the lysosomal escape of MnCO$_3$-ICG. The blue, green, and red colors indicate cell nucleus, ICG, and lysosome, respectively (scale bar, 50 μm). D CLSM imaging of the MnCO$_3$-ICG penetration after different treatment (pH 6.5 with laser or pH 7.4 without laser) in MCSs (scale bar, 200 μm). E Corresponding fluorescence profiles in D. F Calcein-AM and PI co-stained 4T1 cells with different concentration of MnCO$_3$-ICG with or without 808 nm laser (0.5 W/cm$^2$, 5 min) irradiation, the green and red fluorescence indicate live cells and dead cells. G Cell viabilities of 4T1 cells measured by MTT assays, after incubating with different concentration of MnCO$_3$-ICG with or without 808 nm laser (0.5 W/cm$^2$, 5 min) irradiation. H Calcein-AM and PI co-stained 4T1 MCSs after different treatment for three days (PBS, MnCO$_3$-ICG with [Mn]: 20 μg/mL, 50 μg/mL without and with laser irradiation (808 nm, 0.5 W/cm$^2$, 5 min)). I Flow cyogram representing apoptosis assay based on Annexin V-FITC and propidium iodide staining of 4T1 cells after treatment with different therapeutic groups. J Early apoptosis (V+/-P−) and K late apoptosis (V+/-P+) of the 4T1 cells after treatment with different therapeutic groups.
cytoplasm by prolonging the incubation time (Fig. 2A). The uptake profile was also verified by flow cytometry (Fig. 2B). Notably, such MnCO₃-ICG exhibited time-dependent cell internalization profiles via endocytosis pathways as evidenced by the colocalization of the fluorescence of ICG fluorescence with that of LysoTracker (Fig. 2C). The colocalization of ICG and lysosome at 24 h post-incubation was 0.46 ± 0.050 by Pearson’s correlation via ImageJ analysis. To find out whether the MnCO₃-ICG might result in enhanced penetration, we used the multicellular spheroids (MCSSs) derived from 4T1 cells as an in vitro model. The MCSSs were treated with MnCO₃-ICG under different incubation environment (pH 6.5 with laser or pH 7.4 without laser) for 24 h, and the penetration process was measured by using confocal microscopy. MnCO₃-ICG irradiated by laser under slightly acidic environment leaded to better cellular uptake, when compared to control group (Fig. 2D, E).

The in vitro anti-tumor efficiency of MnCO₃-ICG was evaluated in multiple ways, including calcein-AM and propidium iodide (PI) staining, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay, flow cytometry and MCSSs. As a result, we found that treatment of only MnCO₃-ICG results in significant concentration-dependent cell death as indicated by the live-dead dual staining using calcein-AM and PI; the most effective cell death was induced by the treatment of MnCO₃-ICG plus laser exposure (Fig. 2F), which was confirmed by the existence of only red fluorescence in the cells. As expected, MnCO₃-ICG displayed a dose- and time-dependent toxicity to 4T1 cells. Lower concentrations of MnCO₃-ICG had only minor harm to 4T1 cells, and higher concentrations of MnCO₃-ICG demonstrated a rapid increase in cytotoxicity; laser exposure could further enhance the cytotoxicity (Fig. 2G). Flow cytometry apoptosis technique was also applied to precisely analyze cell apoptosis rate (Fig. 2I–K). Furthermore, we evaluated anticancer efficacy against 4T1 3D MCSSs by AM-PI staining in vitro (Fig. 2H). A decreasing number of live cells (represented by green color) and a reduced size of 3D MCSSs was observed in MnCO₃-ICG and MnCO₃-ICG with laser group, which indicated the excellent anti-cancer efficacy. These results could be explained by excessive ROS-induced oxidative stress. Note that the remarkable killing effect was not limited by tumor types because the MnCO₃-ICG could efficiently destroy multiple types of human tumor cells, including liver cancer and glioma (Additional file 1: Fig. S9A, B); normal human cells managed to tolerate the adverse influence of MnCO₃-ICG with much higher cell viabilities than tumor cells under the same treatment (Additional file 1: Fig. S10). This higher tolerance was attributed to the presence of a sufficient amount of catalase to prevent the normal cells from entering an oxidative stress state caused by excessive ROS [70, 71].

To compare the treatment efficacy, we next calculated the half maximal inhibitory concentration (IC₅₀) of different treatments and tumor cellular lines. Using 4T1 as an example, we found the IC₅₀ of MnCO₃-ICG (11.2 ± 1.0 μg/mL) to be 5.8-fold lower than that of Mn-ICG (64.5 ± 1.3 μg/mL). The results confirmed that in situ self-generation of HCO₃⁻, The decomposition of MnCO₃-ICG, is an important and indispensable condition for Mn-mediated Fenton-like reactions. This in turn induces enhanced chemodynamic therapy.

**In vitro oxidative stress generation of MnCO₃-ICG**

Because of the higher efficacy of anti-tumor efficiency of MnCO₃-ICG, we next investigated the cellular ROS production and anticancer effect in vitro. 4T1 cancer cells were incubated with various concentrations of MnCO₃-ICG, and then stained with DCFH-DA (ROS indicator). The confocal images of 4T1 cells showed that those cells treated with a high concentration of MnCO₃-ICG showed much stronger green fluorescence of DCFH-DA, suggesting a concentration-dependent ROS generation induced by MnCO₃-ICG (Fig. 3A). Interestingly, with the same incubation time and concentration of Mn in Mn-ICG, we observed obviously weak green fluorescence of DCFH-DA (Additional file 1: Fig. S11). These further confirmed that the in situ self-generated HCO₃⁻ ions from the decomposition of MnCO₃-ICG accelerated the Mn-catalyzed decomposition of H₂O₂ and peroxidation reactions [5, 27]. For instance, at a concentration of 20 μg/mL of Mn²⁺, quantitative flow cytometry showed that the fluorescence of DCFH-DA in MnCO₃-ICG was 14.7-fold higher than that of PBS group (Fig. 3B).

The intracellular lipid peroxidation levels were evaluated using BODIPY™ 581/591 C11 (lipid peroxidation sensor) and malondialdehyde (MDA) assay kits. After treatments, the levels of lipid peroxidation increased to 145.0 ± 5.1%, and 101.8 ± 16.7% (Additional file 1: Fig. S12) for MnCO₃-ICG ([Mn]: 20 μg/mL) and PBS, respectively. The increase of fluorescence in the plasma membranes revealed that incubation of 4T1 cells with MnCO₃-ICG successfully led to lipid peroxidation (Fig. 3C). Quantitative analysis showed that the fluorescence of BODIPY-C11 in the 4T1 cells after incubation with MnCO₃-ICG was 1.5- and 3.0-fold higher than that of Mn-ICG and cells only (Additional file 1: Fig. S13), respectively. Actually, 93.5% lipid peroxide efficiency was confirmed by flow cytometry (Fig. 3D). These observations were immediate evidence that the in situ
self-supplying bicarbonates efficiently amplified intracellular oxidative stresses in a Mn-mediated Fenton-like reaction using MnCO$_3$-ICG.

The mitochondria are a primary site of energy production and the site of apoptosis. Mitochondrial dysfunction is a distinctive feature of apoptosis, including loss of mitochondrial membrane potential [72]. Consequently, we measured the changes in the mitochondrial membrane potential of tumor cells using a JC-1 assay kit, in which red fluorescence indicates healthy mitochondria and green fluorescence indicates mitochondria in poor health [73]. According to the CLSM images (Fig. 3E), the red fluorescence was sharply switched to green when increasing the concentration of MnCO$_3$-ICG, and completely switched to green at the highest concentration of Mn (i.e. 20 μg/mL). Flow cytometric analyses also revealed rapid decrease of mitochondrial membrane potential by MnCO$_3$-ICG (Fig. 3F). In comparison, there was little red fluorescence that switched to green in Mn-ICG (Additional file 1: Fig. S14). Lactate dehydrogenase (LDH) is an intracellular active enzyme and was released into the cell culture medium [74, 75], indicating that the cell walls were breached (Fig. 3G; Additional file 1: Fig. S15) [76]. These data suggest a highly negative mitochondrial membrane potential due to the introduction of bicarbonates that induce amplified intracellular oxidative stresses of Mn species [77].

In vivo biodistribution and MR/FL-dual mode imaging

The in vivo pharmacokinetic profiles of MnCO$_3$-ICG ([Mn]: 2 mg/kg) were carefully assessed by detecting the distribution of Mn in 4T1 tumor-bearing mice. Blood circulation profiles followed a classical two-compartment model with the first half-time and second half-time determined to be 1.5 ± 0.5 h and 12.3 ± 3.5 h, respectively (Fig. 4A). We found that MnCO$_3$-ICG passively accumulated into the tumor area and reached the highest level at
Fig. 4 The Distribution of the MnCO$_3$-ICG Complexes on 4T1 Tumor-bearing Mice. A Time course of blood levels of MnCO$_3$-ICG levels following intravenous injection. B In vivo MRI images and (D) fluorescence images of BALB/C tumor-bearing mice taken at different time points after injection of MnCO$_3$-ICG. C Quantification analysis of MRI signal change in tumor/muscle based on region of interest (ROI) analysis on images from panel B. E Quantification analysis of the tumor ratio of fluorescence signal change in tumors based on ROI from panel D. F Biodistribution of Mn (% injected dose (ID) Mn per gram tissue) in main tissues and tumors after intravenous administration of MnCO$_3$-ICG. G Thermal images and (H) real-time temperature curve of BALB/C tumor-bearing mice treated with MnCO$_3$-ICG and 808 nm laser (0.5 W/cm$^2$) irradiation.
4 h post-injection under a 9.4 T in vivo MRI scanner system (Fig. 4B, C). The results showed a relatively high longitudinal relaxivity ($r_1 = 4.3 \text{ mM}^{-1} \text{s}^{-1}$) (Additional file 1: Fig. S16). In addition, fluorescence imaging was also conducted on 4T1 tumor mice, and showed similar results (Fig. 4D, E). By detecting the Mn in organs using ICP-MS, we found the maximum tumor accumulation of MnCO$_3$-ICG to be $23.7 \pm 4.7\%\text{ID/g}$ (percent injected dose per gram tissue). This remained as high as $8.8 \pm 0.9\%\text{ID/g}$ at 12 h post-injection (Fig. 4F). The ex vivo fluorescence imaging (Additional file 1: Fig. S17) at 24 after intravenous injection showed an obvious signal in the tumor. The signal intensity of MR and fluorescence in tumor regions remained strong even at the 24 h post injection, which indicated the accumulation of Mn$^{2+}$ into the tumor site for a long time to offer desirable and long-term CDT efficacy. At 4 h post-injection when the accumulation reached the highest level, we irradiated the tumor area with 808 nm laser (0.5 W/cm$^2$). The temperature increased sharply by about 10 °C within 2 min and remained at that temperature for 5 min (Fig. 4G, H).

Next, we carefully evaluated the in vivo therapeutic efficacy of MnCO$_3$-ICG with or without laser irradiation by using 4T1 tumor mice. When the tumor volume reached about 60 mm$^3$, the mice were divided into six groups ($n = 5$/group) (Fig. 5A): (Group 1) PBS (four doses); (Group 2) MnCO$_3$-ICG ([Mn]: 2 mg/kg, four doses, termed as CDT-1); (Group 3) 2 × MnCO$_3$-ICG ([Mn]: 4 mg/kg, four doses, termed as CDT-2); (Group 4) 4 × MnCO$_3$-ICG ([Mn]: 8 mg/kg, four doses, termed as CDT-3); (Group 5) MnCO$_3$-ICG ([Mn]: 2 mg/kg + L (0.5 W/cm$^2$), one dose, termed as PTT); and (Group 6) MnCO$_3$-ICG ([Mn]: 2 mg/kg + L (0.5 W/cm$^2$), three doses, termed as PTT + CDT). The laser (808 nm, 0.5 W/cm$^2$, 5 min) was applied to the tumors at 4 h post-injection of MnCO$_3$-ICG. We found that the tumors on the mice of Groups 4, 5 and 6 were more effectively suppressed over those in the other Groups (Fig. 5B; Additional file 1: S18). The tumors in Group 5 recurred towards the ending point, however, there was no recurrence even after 21 days post-treatment in Group 6 (Fig. 5B, C).

**In vivo therapeutic efficacy of MnCO$_3$-ICG for subcutaneous tumor**

To evaluate the therapy efficacy, we sacrificed all mice in Groups 1–5 and randomly selected two mice in Group 6 to measure the tumor weight. The weights and photographs were consistent with the statistical analysis of tumor size (Fig. 5C; Additional file 1: Fig. S19). The statistical analysis showed that the tumor growth inhibition (TGI) rates were 49.0% (Group 2), 63.8% (Group 3), and 76.3% (Group 4) (Fig. S20). Remarkably, Group 6 resulted in a complete tumor inhibition (Fig. S20 and S21). The hematoxylin and eosin (H&E) images (Fig. 5D) and immunofluorescence staining of vessel (Additional file 1: Fig. S22) proved that the tumor in the PTT group had a complete tumor tissue and vessel structure, which was prerequisite and foundation of MnCO$_3$-ICG accumulation to the tumor after irradiation. Furthermore, the H&E staining of tumor slices collected from the different treatment mice exhibited the most severe histological damages (i.e. no tumor modules were found) versus those with no treatment (Fig. 5D).

In vivo safety evaluation of MnCO$_3$-ICG complexes

The biocompatibility of MnCO$_3$-ICG was evaluated with the 100% tumor growth inhibition was a result of CDT&PTT induced immunogenic cell death combined with novel adjuvants (Mn$^{2+}$) activating innate immunity [17, 54, 55]. Herein, to further elucidate the enhanced anti-tumor immunity afforded by Mn$^{2+}$ adjuvant strategy, the in vivo immunogenic effect was evaluated by testing the dendritic cells (DCs) maturation and T cells activation of mice spleen after treatment, as well as the major cell cytokines in serum from a portion of the tested mice. In addition, the dramatic changes of IL-6, IL-1α and TNF-α in serum were observed after different treatments (Fig. 5F), which indicated MnCO$_3$-ICG-mediated acute inflammatory response. As shown in Fig. 5G, the percentage of mature DCs in group 6 (PTT & CDT) was much higher than that in the control. Compared with mice treated with CDT alone or PTT alone, combination therapy strategy showed lots of CD8$^+$ cytotoxic T lymphocytes (CTLs) recruitment, indicating the effective activation of innate immune response (Fig. 5H; Additional file 1: Fig. S24). These results indicated that MnCO$_3$-ICG had a potential to activate immunotherapeutic efficacy, due to the use of immune adjuvants.

**In vivo immunogenic effect evaluation of combination therapy strategy**

We speculated that the 100% tumor growth inhibition was a result of CDT&PTT induced immunogenic cell death combined with novel adjuvants (Mn$^{2+}$) activating innate immunity [17, 54, 55]. Herein, to further elucidate the enhanced anti-tumor immunity afforded by Mn$^{2+}$ adjuvant strategy, the in vivo immunogenic effect was evaluated by testing the dendritic cells (DCs) maturation and T cells activation of mice spleen after treatment, as well as the major cell cytokines in serum from a portion of the tested mice. In addition, the dramatic changes of IL-6, IL-1α and TNF-α in serum were observed after different treatments (Fig. 5F), which indicated MnCO$_3$-ICG-mediated acute inflammatory response. As shown in Fig. 5G, the percentage of mature DCs in group 6 (PTT & CDT) was much higher than that in the control. Compared with mice treated with CDT alone or PTT alone, combination therapy strategy showed lots of CD8$^+$ cytotoxic T lymphocytes (CTLs) recruitment, indicating the effective activation of innate immune response (Fig. 5H; Additional file 1: Fig. S24). These results indicated that MnCO$_3$-ICG had a potential to activate immunotherapeutic efficacy, due to the use of immune adjuvants.

**In vivo safety evaluation of MnCO$_3$-ICG complexes**

The biocompatibility of MnCO$_3$-ICG was evaluated by recording the body weight of treated mice throughout the entire treatment process and the histological...
In vivo therapeutic efficacy of MnCO$_3$-ICG for orthotopic hepatocellular carcinoma

Due to the excellent therapeutic effect and immune response, we further evaluated the anticancer activity of MnCO$_3$-ICG in an orthotopic hepatocellular carcinoma (Hep 1–6) model. Flow cytometry assay revealed a significant dose-dependent increase of the Hep 1–6 cell apoptosis upon treatment (Fig. 6A). We also evaluated the oxidative stress response of Hep 1–6 tumor cells through the generation of ROS (Additional file 1: Fig. S27) and the level of lipid peroxidation (Additional file 1: Fig. S28). The obvious increase of fluorescence signal indicating MnCO$_3$-ICG can induce sharp cellular oxidative stress. Mitochondrial and cell membrane damage induced by oxidative stress was confirmed by JC-1 assay (Additional file 1: Fig. S29) and LDH assay kit (Additional file 1: Fig. S30).

Therefore, orthotopic hepatocellular carcinoma therapy of MnCO$_3$-ICG was conducted on Hep 1–6-tumor-bearing mice. To minimize photothermal-mediated normal liver tissue damage, distributing pattern of MnCO$_3$-ICG between normal liver and tumor was studied using magnetic resonance imaging. The MR signal intensity in liver and tumor gradually increased after MnCO$_3$-ICG injection (Fig. 6B). There were significant different signal changes between normal liver and tumor after treatment for 8 h, although the hepatic uptake was not the highest at this time (Fig. 6C, D). The therapeutic efficacy of MnCO$_3$-ICG was evaluated after confirming the establishment of tumors by bioluminescence imaging (BLI). The tumor-bearing mice were divided randomly into 3 groups (n=5/group): (1) PBS; (2) MnCO$_3$-ICG (2 mg/kg, three doses, termed as CDT); (3) MnCO$_3$-ICG ([Mn]:2 mg/kg+L (0.5 W/cm$^2$), three doses, termed as PTT+CDT). Three injections were administrated intravenously on days 0, 3 and 6, and the laser (808 nm, 0.5 W/cm$^2$, 5 min) was applied to the tumors at 8 h post-1st-injection of MnCO$_3$-ICG. During the treatment, the orthotopic hepatic tumor growth was monitored by BLI. The fluorescence intensity of group 3 remained unchanged or even decreased slightly, which was a sharp contrast to the increase of fluorescence intensity in the control group (Fig. 6E). Combination immunotherapy strategy significantly inhibited tumor growth (TGIs on day 12 were 54.2% (Group 2) and 79.4% (Group 3)). To further study the therapeutic efficacy, livers on mice after various treatments were harvested, photographed, and analyzed by H&E staining (Fig. 6F–H). Significant neoplasms were observed in the liver tissue of the control group. Severely damaged structural disruptions and pathological changes were observed in tiny nodules of tumor after treatment. H&E staining of livers indicating the safety of MnCO$_3$-ICG, even with the laser for hepatocellular carcinoma in situ. In brief, these results demonstrated that MnCO$_3$-ICG-mediated PTT&CDT combined immunotherapy strategy could serve as a potential therapy for the elimination of tumor in situ.

Conclusion

In summary, we proposed a self-supplying bicarbonate- and Mn-pool Fenton-like MnCO$_3$-ICG catalyst with amplification of tumor oxidative stress and regulation of intracellular pH. ICG molecules were employed as coordination sites to regulate the decomposition level in the acidic environment. MnCO$_3$-ICG degraded and released HCO$_3^−$ and Mn$^{2+}$ in situ, and the increased HCO$_3^−$ inside the cells could escalate Mn-mediated Fenton-like reaction to accelerate the generation of •OH for oxidative stress damage of tumors cells. The evaluation of cellular internalization in vitro showed that MnCO$_3$-ICG could be internalized by cells and released from lysosome to cytosols, in which Mn$^{2+}$ had more opportunities to generate cellular oxidative stress by reacting with H$_2$O$_2$ in the presence of HCO$_3^−$. The in vivo results confirmed that the combination of CDT, PTT combined with nanoadjuvant (Mn$^{2+}$)-enhanced immunotherapy effectively suppressed the tumor growth, and realized complete tumor elimination. The combination therapy strategy promotes DC maturation, enhances the CTL-mediated cytotoxic effect, and generates multiple pro-inflammatory factors.
Fig. 5 (See legend on previous page.)
Fig. 6 The Antitumor Efficiency for Hepatocellular Carcinoma (Hep 1–6) Cells and Orthotopic Hepatic Tumors. A Flow cytogram representing apoptosis assay based on Annexin V-FITC and propidium iodide staining of 4T1 cells after treatment with different therapeutic groups. B In vivo T₁/T₂ MRI images for orthotopic hepatic tumor-bearing mice taken at different time points after injection of MnCO₃-ICG. Quantification analysis of MRI signal change in C tumor/noise and D tumor/liver based on region of interest (ROI) analysis on images from panel B. E Relative luminescence intensity changes based on the bioluminescence images (BLI). F BLI changes after different treatments duration of therapy. G Photographs of representative tumors and H H&E-stained tumor sections taken from tumor-bearing mice after various treatments (scale bar: 200 μm)
(IL-6, IL-1α and TNF-α). This study provides a potential synergistical strategy to improve the therapeutic effect for orthotopic tumors by releasing “ion drug” in an on-demand manner.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12951-022-01404-x.

Additional file 1: Figure S1. Typical TEM images of MnCO3-ICG complexes prepared at different feeding ratios of ICG and MnCO3·H2O. Figure S2. (a) the absorption spectra of MnCO3-ICG complexes at different time. (b) Typical TEM images of MnCO3-ICG complexes prepared at different time. Figure S3. (a) TEM imaging of MnCO3-ICG-PAH complexes prepared with different feeding ratios of MnCO3-ICG and PAH. (b) Photographs of MnCO3·H2O-ICG-PAH complexes after being incubated in H2O, PBS (7.4), cell medium (DMEM), and serum (FBS) for 12 h. (c) The hydrodynamic diameter of MnCO3·H2O-ICG-PAH complexes with different feeding ratios. Figure S4. (a) X-ray powder diffraction pattern of the MnCO3-ICG-anion re-assembled nanoformulation after incubation in the acidic buffer solutions. (b) XPS spectra for MnCO3-ICG and the re-assembled nanoformulation after incubation in the acidic buffer solutions. XPS spectra of Mn2p for (c) MnCO3-ICG and (d) the re-assembled nanoformulation after incubation in the acidic buffer solutions. Figure S5. (a) Ultrasonic images of the generation of CO2 after CaCO3 incubation with different time (pH 5.8±H2O2). (b) Ultrasonic signal intensity of the generation of CO2 after CaCO3 incubation with different time (pH 5.8±H2O2). (c) The typical chromatograms of CO2 generated after MnCO3-ICG incubation with acidic environment. Figure S6. (a) Colorimetric analysis of the Fenton-like reaction for MB decolorization with different concentration of H2O2 — in acidic environment. (b) Colorimetric analysis of the Fenton-like reaction for MB decolorization with different treatment. (c) Colorimetric analysis of the Fenton-like reaction for MB decolorization after different reaction time with acidic environment (pH 5.8). Figure S7. (a) Colorimetric analysis of the Fenton-like reaction for MB decolorization after pulling a vacuum. (b) Bar plot showing the of MB after different treatments. Figure S8. The absorption spectra of ICG, Mn-ICG and MnCO3-ICG. Figure S9. (a) Hep G2 cells and (b) U87/MG cells incubated with different concentrations of MnCO3-ICG with or without 808 nm laser (0.5 W/cm2, 5 min) irradiation. Figure S10. Relative cellular viabilities of normal L02 and 3T3 cells incubated with different concentrations of MnCO3-ICG. Figure S11. Intracellular -OH generation after incubation with Mn-ICG detected by DCFH-DA probe (scale bar, 50 μm). Figure S12. Lipid damage assessment measured by lipid peroxidation assays (p < 0.05). Figure S13. (a) CLSM observation on the intracellular distribution of lipoperoxides in 4T1 cells after incubation with PBS and MnCO3-ICG for 24 h. The red fluorescence is the lipid ROS in cells and membranes after the staining with BODIPY-C11 (scale bar, 50 μm). (b) Lipoperoxides, based on BODIPY staining results in panel (a). Figure S14. (a) CLSM observation on the changes in the mitochondrial membrane potential of 4T1 cells after incubation with different concentration of Mn-ICG complexes prepared at different time. (b) The membrane potential (ΔΨm) changes, assessed by JC-1 staining (***p < 0.001). Figure S15. LDH release assay of Hep 1–6 cells after incubation with different concentration of MnCO3-ICG (scale bar, 50 μm). (b) The membrane potential (ΔΨm) changes, assessed by JC-1 staining (**p < 0.01). Figure S16. T1-relaxation rate (T1) and T2-weighted MR images of MnCO3-ICG. Figure S17. Ex vivo fluorescence images of the organs harvested in BALB/c tumor-bearing mice at 24 h post-injection. Figure S18. Body and tumor weights of 4T1 cells and mice treated after the 14-day treatment period. Groups 1, 2, 3, 4, 5 and 6 were used to represent PBS, MnCO3-ICG (one dose) and MnCO3-ICG (two doses), respectively. Figure S19. Images of representative tumors taken from mice in different formulations after the 14-day treatment period. Groups 1, 2, 3, 4, 5 and 6 were used to represent PBS, MnCO3-ICG (one dose) and MnCO3-ICG (two doses), respectively. Figure S20. The tumor growth inhibition curves of BALB/C tumor-bearing mice exposed to different formulations after the treatment period. Groups 1, 2, 3, 4, 5 and 6 were used to represent PBS, MnCO3-ICG (one dose) and MnCO3-ICG (two doses), respectively.

Acknowledgements
We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

Author contributions
YF and HC conceived and designed the research; YF performed the synthesis; YF and YL carried out the characterization experiments; XM and YF performed the cell experiment. DD, LC and ZW performed the in vivo experiments; LX, RQ and YL analyzed the data; YF, WS and HC co-wrote the paper. All authors read and approved the final manuscript.

Funding
The work was supported by the National Natural Science Foundation of China (82172007, 81771977, 82001956), the Science Fund for Distinguished Young Scholars of Fujian Province (2021J06007), the National Postdoctoral Program for Innovative Talents (2020020196), the Xiamen Science and Technology Plan Project (3502Z202103017), the Fundamental Research Funds for the Central Universities of China (2021J06007), the open research fund of National Facility for Translational Medicine (Shanghai) (TMSK-2021-102). All animal experiments were approved by the Animal Management and Ethics Committee of the Xiamen University.
Availability of data and materials
The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
All animal experiments were approved by the Animal Management and Ethics Committee of the Xiamen University.

Consent for publication
All authors agree to be published.

Competing interests
The authors declare no conflict of interests.

Received: 26 February 2022   Accepted: 30 March 2022
Published online: 19 April 2022

References
1. Tang Z, Liu Y, He M, Bu W. Chemodynamic therapy: tumour microenvironment-mediated Fenton and Fenton-like reactions. Angew Chem, Int Ed. 2019;58:946–56.
2. Liu J, Chen Q, Feng L, Liu Z. Nanomedicine for tumor microenvironment modulation and cancer treatment enhancement. Nano Today. 2018;12:55–73.
3. Fan K, Gao C, Pan Y, Li D, Yang D, Feng J, Song L, Liang M, Yan X. Magnetotetramericin nanoparticles for targeting and visualizing tumour tissue. Nat Nanotechnol. 2012;7:459–64.
4. Gao L, Zhang J, Nie L, Zhang J, Yang G, Wu N, Wang T, Feng J, Yang D, Perrett S, Yan X. Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. Nat Nanotechnol. 2007;2:577–83.
5. Lin LS, Song J, Song L, Ke K, Liu Y, Zhou Z, Shen Z, Li J, Yang Z, Tang W, et al. Simultaneous Fenton-like ion delivery and glutathione depletion by MnO2-based nanoagent to enhance chemodynamic therapy. Angew Chem Int Ed. 2018;57:4902–6.
6. Pignatelto JJ, Olevier E, MacKay A. Advanced oxidation processes for organic contaminant destruction based on the Fenton reaction and related chemistry. Crit Rev Environ Sci Technol. 2006;361–84.
7. Tan J, Li H, Hu X, Abdullah R, Xie S, Zhang L, Zhao M, Luo Q, Li Y, Sun Z, et al. Size-tunable assemblies based on Ferrocene-containing DNA polymers for spatially uniform penetration. Chem. 2019;5:1775–92.
8. Fridovich I. The biology of oxygen radicals. Science. 1978;198:875–80.
9. Winterbourn CC. Reconciling the chemistry and biology of reactive oxygen species. Nat Chem Biol. 2008;4:278–86.
10. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition. 2002;18:887–92.
11. Zheng G, Chen J, Steffková K, Jarv M, Li H, Wilson BC. Photodynamic molecular beacon as an activatable photosensitizer based on protease-controlled single oxygen quenching and activation. Proc Natl Acad Sci USA. 2007;104:8989–94.
12. Stockwell BR, Friedrichs Angelner JP, Bayli H, Bush AL, Conrad M, Dixon SJ, Fulda S, Gaason S, Hatzios SK, Kagan VE, et al. Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. Cell. 2017;171:273–85.
13. Tarangelo A, Dixon SJ. Nanomedicine: an iron age for cancer therapy. Nat Nanotechnol. 2016;11:921–2.
14. Kim SE, Zhang L, Ma K, Riegman M, Chen F, Ingold K, Conrad M, Turkul MZ, Gao M, Jiang X, et al. Ultrasmall nanoparticles induce ferroptosis in nutrient-deprived cancer cells and suppress tumour growth. Nat Nanotechnol. 2016;11:797–85.
15. Ding B, Zheng P, Jiang F, Zhao Y, Wang M, Chang M, Ma P, Lin J. MnO nanospikes as nanoadjuvants and immunogenic cell death drugs with enhanced antimicrobial activity and antibacterial transformation. Angew Chem Int Ed. 2020;59:16381–4.
16. Hou L, Tian C, Yan Y, Zhang L, Zhang H, Zhang Z. Manganese-based nanocatalyst optimizes cancer immunotherapy via enhancing innate immunity. ACS Nano. 2020;14:3927–40.
17. Lv M, Chen M, Zhang R, Zhang W, Wang C, Zhang Y, Wei X, Guan Y, Liu J, Feng K, et al. Manganese is critical for antitumor immune responses via cGAS-STING and improves the efficacy of clinical immunotherapy. Cell Res. 2020;30:966–79.
18. Shin J, Anisur BM, Ko MK, Im GH, Lee JH, Lee IS. Hollow manganese oxide nanoparticles as multifunctional agents for magnetic resonance imaging and drug delivery. Angew Chem Int Ed. 2009;48:321–4.
19. Wang Z, Zhang Y, Jie E, Liu Z, Cao F, Chen Z, Ren J, Xu Q. Biomimetic nanoflowers by self-assembly of nanozymes to induce intracellular oxidative damage against hypoxic tumors. Nat Commun. 2018;9:3334.
20. Fan H, Yan G, Zhao Z, Hu X, Zhang W, Liu H, Fu X, Fu T, Zhang XB, Tan W. A smart photosensitizer-manganese dioxide nanosystem for enhanced photodynamic therapy by reducing glutathione levels in cancer cells. Angew Chem Int Ed. 2016;55:5477–82.
21. Qian X, Han Y, Xu L, Xu T, Chen Y. Manganese-based functional nanoplatforms: nanosynthetic construction, physiochemical property, and theranostic applicability. Adv Funct Mater. 2019;30:1907066.
22. Liu C, Wang D, Zhang S, Cheng Y, Yang F, Xing Y, Xu T, Dong H, Zhang X. Biodegradable biomimetic Copper/Manganese Silicate nanospheres for chemodynamic/photodynamic synergistic therapy with simultaneous glutathione depletion and hypoxia relief. ACS Nano. 2019;13:4267–77.
23. Lisher PJ. Probing manganese homeostasis and the oxidative stress response in Streptococcus pneumoniae. Indiana University, 2016.
24. Dong Z, Feng L, Hao Y, Chen M, Gao M, Chao Y, Zhao H, Zhu W, Liu J, Liang C, et al. Synthesis of hollow biominalerized CaCO3-polydopamine nanoparticles for multimodal imaging-guided cancer photodynamic therapy with reduced skin photosensitivity. J Am Chem Soc. 2018;140:2165–78.
25. Dong Z, Feng L, Hao Y, Li Q, Chen M, Yang Z, Zhao H, Liu J. Synthesis of CaCO3-based nanomende for enhanced sonodynamic therapy via amplification of tumor oxidative stress. Chem. 2020;6:1391–407.
26. Cheng Y, Zhang S, Kang N, Huang J, Lu X, Wei K, Ye S, Chen Z, Zhou X, Ren L. Polydopamine-coated manganese carbonate nanoparticles for amplified magnetic resonance imaging-guided photothermal therapy. ACS Appl Mater Interfaces. 2017;9:19296–306.
27. Wang P, Liang C, Zhu J, Yang N, Jiao A, Wang W, Song X, Dong X. Manganese-based nanoplatform as metal ion-enhanced ROS generator for combined chemodynamic/photodynamic therapy. ACS Appl Mater Interfaces. 2019;11:141140–7.
28. Thomsen HS, Barentsz JO, Burchart C, Chabanova E, Dekker HM, Moesgaard F, Moller JM, Leth-Espensen P, Logager V, Takahashi S. Initial clinical experience with oral manganese (CMC-001) for liver MR imaging. Eur Radiol. 2007;17:273–8.
29. Thomsen HS, Svendsen O, Klarup S. Increased manganese concentration in the liver after oral intake. Acad Radiol. 2004;11:38–44.
30. Bartolozzi C, Donati F, Cioni D, Crocetti L, Lencioni R. MnDPDP-enhanced MRI vs dual-phase spiral CT in the detection of hepatocellular carcinoma in cirrhosis. Eur Radiol. 2000;10:1697–702.
31. Low RR. Contrast agents for MR imaging of the liver. Clin J Magn Reson Imaging. 1997;5:56–67.
32. Cheng Y, Yang F, Zhang K, Zhang Y, Cao Y, Liu C, Lu H, Dong H, Zhang X. Non-Fenton-type hydrogen radical generation and photothermal effect by mitochondria-targeted WSe2/MnO2 nanocomposite loaded with isoniazid for synergistic anticancer treatment. Adv Funct Mater. 2019;29:1903850.
33. Liochev SI, Fridovich I. Carbon dioxide mediates Mn(II)-catalyzed decomposition of hydrogen peroxide and peroxidation reactions. Proc Natl Acad Sci USA. 2004;101:12485–90.
34. Berlett BS, Chock PB, Yim MB, Stadtman ER. Manganese catalyzes the bicarbonate-dependent oxidation of amino acids by hydrogen peroxide and the amino acid-facilitated dismutation of hydrogen peroxide. Proc Natl Acad Sci USA. 2004;101:12485–90.
35. Berlett BS, Chock PB, Stadtman ER. Manganese(II)-bicarbonate-mediated catalytic activity for hydrogen peroxide dismutation. Proc Natl Acad Sci USA. 1990;87:394–8.
36. Stadtman ER, Berlett BS, Chock PB. Manganese-dependent disproportionation of hydrogen peroxide in bicarbonate buffer. Proc Natl Acad Sci USA. 1990;87:384–8.
37. Lane BS, Vogt M, DeRose VJ, Burgess K. Manganese-catalyzed epoxidations of alkenes in bicarbonate solution. J Am Chem Soc. 2002;124:1946–54.
38. Ember E, Rothbart S, Puchta R, Eldik Rv. Metal ion-catalyzed oxidative degradation of Orange II by H₂O₂. High catalytic activity of simple manganese salts. New J Chem. 2009;33:34–49.
39. Meng ZH, Wu SH, Sun SW, Xu Z, Zhang XC, Wang XM, Liu Y, Ren HF, Jia SY, Bai H. Han X. Formation and oxidation reactivity of MnO₂·(HCO₃)₂ in the MnO₂·(HCO₃)₂·H₂O system. Inorg. Chem. 2020;59:3171–80.
40. Casey JR, Grinstein S, Orlovski I. Sensors and regulators of intracellular pH. Nat Rev Mol Cell Biol. 2010;11:50–61.
41. Pastorekova S, Parkkila S, Pastorek J, Supuran CT. Carbonic anhydrases: current state of the art, therapeutic applications and future prospects. J Enzyme Inhib. 2004;19:199–229.
42. Stewart AK, Boyd CAR, Vaughan-Jones RD. A novel role for carbonic anhydrase: cytoplasmic pH gradient dissipation in mouse small intestinal enterocytes. J Physiol. 2002;239:81–91.
43. Ke CJ, Su TY, Chen HL, Liu HL, Chiang WL, Chu PC, Xia Y, Sung HW. Smart multifunctional hollow microspheres for the quick release of drugs in intracellular lysosomal compartments. Angew Chem Int Ed. 2011;50:886–9.
44. Choi BY, Park HJ, Hwang SJ, Park JB. Preparation of alginate beads for floating drug delivery system effects of CO₂-generating agents. Int J Pharm. 2002;239:81–91.
45. Hulikova A, Vaughan-Jones RD, Swietach P. Dual role of CO₂/HCO₃⁻ buffer in regulation of intracellular pH of three-dimensional tumor growths. J Biol Chem. 2011;286:13815–26.
46. Hao G, Xu Z, Li L. Manipulating extracellular tumor pH: an effective target for cancer therapy. RSC Adv. 2018;8:21882–92.
47. Pilon-Thomas S, Kodumudi KN, El-Kenawi AE, Russell S, Weber AM, Luddy K, Damaghi M, Wojtkowiak JW, Mule JJ, Ibrahim-Hashim A, Gelles RJ. Neutralization of tumor acidity improves antitumor responses to immunotherapy. Cancer Res. 2016;76:1381–90.
48. Yang M, Zhong X, Yuan Y. Does baking soda function as a magic bullet for patients with cancer? A mini review. Int Cancer Ther. 2020;15:437345202002579.
49. Chao M, Wu H, Jin K, Li B, Wu J, Zhang G, Yang H, Xu X. A nonrandomized cohort and a randomized study of local control of large hepatocarcinoma by targeting intratumoral lactic acidosis. Elife. 2016;5:e15691.
50. Takayasu K, Arii S, Ikai I, Omata M, Okita K, Khida T, Matsumiya Y, Nakamura Y, Kojoito M, Makuuchi M, Yamaoka Y. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8110 patients. Gastroenterology. 2006;131:461–9.
51. Corbet C, Feron O. Tumour acidity: from the passenger to the driver’s state of the art, therapeutic applications and future prospects. J Enzyme Inhib. 2007;22:562–74.
52. Zhang H. Will cancer cells be defeated by sodium bicarbonate? Nat Rev Drug Discov. 2021;20:454–75.
53. Wang C, Guan Y, Lv M, Zhang R, Guo Z, Wei X, Du X, Yang J, Li T, Wan Y, et al. Manganese increases the sensitivity of the cGAS-STING pathway for mitochondrial calcium carbonate/doxorubicin/silica nanoreactor for pH-responsive delivery of an anticancer drug. Angew Chem Int Ed. 2015;54:919–22.
54. Zhou Y, Luo Z, Li M, Qu Q, Ma X, Yu SH, Zhao Y. A preloaded amorphous calcium carbonate/doxorubicin/silica nanoreactor for pH-responsive delivery of an anticancer drug. Angew Chem Int Ed. 2015;54:919–22.
55. Damaghi M, Wojtkowiak JW, Mule JJ, Ibrahim-Hashim A, Gillies RJ. Neutralization of tumor acidity improves antitumor responses to immunotherapy. Cancer Res. 2016;76:1381–90.
56. Kroemer G, Galluzzi L. Mitochondrial membrane permeabilization in cell death. Physiol Rev. 2007;87:99–163.
57. Nakai M, Mori A, Watanabe A, Mitsumoto Y. 1-methyl-4-phenylpyridinium (MPP⁺) decreases mitochondrial oxidation-reduction (REDOX) activity and membrane potential (ΔΨm) in rat striatum. Exp Neurol. 2003;179:103–10.
58. Chen R, Zeng L, Zhu S, Liu J, Zeh HJ, Kroemer G, Wang H, Billiar TR, Jiang J, Tang D, Kang R. cAMP metabolism controls caspase-11 inflammasome activation and pyroptosis in sepsis. Sci Adv. 2019;5:eaav5562.
59. Haslam G, Wyatt D, Kittos PA. Estimating the number of viable animal cells in multi-well cultures based on their lactate dehydrogenase activities. Cytotechnology. 2000;32:63–73.
60. Jiang W, Yin L, Chen H, Paschall AV, Zhang L, Fu W, Zhang W, Todd T, Yu KS, Zhou S, et al. NaCl nanoparticles as a cancer therapeutic. Adv Mater. 2019;31:e1904058.
61. Chao M, Wu H, Jin K, Li B, Wu J, Zhang G, Yang H, Xu X. A nonrandomized cohort and a randomized study of local control of large hepatocarcinoma by targeting intratumoral lactic acidosis. Elife. 2016;5:e15691.
62. Pastorekova S, Parkkila S, Pastorek J, Supuran CT. Carbonic anhydrases: current state of the art, therapeutic applications and future prospects. J Enzyme Inhib. 2004;19:199–229.
63. Stewart AK, Boyd CAR, Vaughan-Jones RD. A novel role for carbonic anhydrase: cytoplasmic pH gradient dissipation in mouse small intestinal enterocytes. J Physiol. 2002;239:81–91.
64. Hulikova A, Vaughan-Jones RD, Swietach P. Dual role of CO₂/HCO₃⁻ buffer in regulation of intracellular pH of three-dimensional tumor growths. J Biol Chem. 2011;286:13815–26.
65. Hao G, Xu Z, Li L. Manipulating extracellular tumor pH: an effective target for cancer therapy. RSC Adv. 2018;8:21882–92.
66. Chao M, Wu H, Jin K, Li B, Wu J, Zhang G, Yang H, Xu X. A nonrandomized cohort and a randomized study of local control of large hepatocarcinoma by targeting intratumoral lactic acidosis. Elife. 2016;5:e15691.
67. Takayasu K, Arii S, Ikai I, Omata M, Okita K, Khida T, Matsumiya Y, Nakamura Y, Kojoito M, Makuuchi M, Yamaoka Y. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8110 patients. Gastroenterology. 2006;131:461–9.
68. Corbet C, Feron O. Tumour acidity: from the passenger to the driver’s state of the art, therapeutic applications and future prospects. J Enzyme Inhib. 2007;22:562–74.
69. Zhang H. Will cancer cells be defeated by sodium bicarbonate? Nat Rev Drug Discov. 2021;20:454–75.
70. Damaghi M, Wojtkowiak JW, Mule JJ, Ibrahim-Hashim A, Gillies RJ. Neutralization of tumor acidity improves antitumor responses to immunotherapy. Cancer Res. 2016;76:1381–90.
71. Kroemer G, Galluzzi L. Mitochondrial membrane permeabilization in cell death. Physiol Rev. 2007;87:99–163.
72. Haslam G, Wyatt D, Kittos PA. Estimating the number of viable animal cells in multi-well cultures based on their lactate dehydrogenase activities. Cytotechnology. 2000;32:63–73.
73. Jiang W, Yin L, Chen H, Paschall AV, Zhang L, Fu W, Zhang W, Todd T, Yu KS, Zhou S, et al. NaCl nanoparticles as a cancer therapeutic. Adv Mater. 2019;31:e1904058.
74. Gu T, Wang Y, Lu Y. A novel medicine fabricated from Gold nanoparticles-decorated metal-organic framework for cascade chemo/chemodynamic cancer therapy. Adv Sci. 2020;7:2001060.
75. Chen Y, Huang Y, Zhou S, Sun M, Chen L, Wang J, Xu M, Liu S, Liang K, Zhang Q, et al. Tailored chemodynamic nanomedicine improves pancreatic cancer treatment via controllable damaging neoplastic cells and reprogramming tumor microenvironment. Nano Lett. 2020;20:6780–90.
76. Kroemer G, Galluzzi L. Mitochondrial membrane permeabilization in cell death. Physiol Rev. 2007;87:99–163.
77. Gu T, Wang Y, Lu Y, Cheng L, Feng L, Zhang H, Li X, Han G, Liu Z. Platinum nanoparticles to enable electrodynamic therapy for effective cancer treatment. Adv Mater. 2019;31:e1806803.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Feng et al. Journal of Nanobiotechnology (2022) 20:193
Page 17 of 17