Employing the thiol-ene click reaction via metal-organic frameworks for integrated sonodynamic-starvation therapy as an oncology treatment

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ABSTRACT Hypoxia in the tumor microenvironment (TME) greatly limits the tumor-killing therapeutic efficacy of sonodynamic therapy (SDT); this phenomenon is further exacerbated by increased glutathione (GSH) levels in cancer cells. Simultaneously, cancer starvation therapy is increasingly recognized nowadays as a promising clinical translation, but the efficacy of glucose oxidase (GOx)-based starvation therapy is also limited by the lack of oxygen in the tumor. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a key glycolytic enzyme and can therefore be a target for starvation therapy in the absence of oxygen engagement. Here, we proposed thiol-ene click reactions based on a two-dimensional metal-organic framework (MOF) modification for tumor treatments to enable the combination of SDT and starvation therapy. Experimental studies demonstrated that the prepared material could consume GSH and GAPDH free from oxygen in TME, which benefited from the thiol-ene click reactions between the MOFs and thiol substances in cancer cells. Further experiments *in vitro* and *in vivo* indicated the prepared MOF materials could kill cancer cells efficiently. This study is expected to create a promising avenue for thiol-ene click reactions in SDT and starvation therapy for cancer.

Keywords: metal-organic framework, sonodynamic therapy, starvation therapy, thiol-ene click reactions

INTRODUCTION

Sonodynamic therapy (SDT) is an attractive therapeutic approach for killing malignant tumors due to its noninvasiveness, site-confined irradiation, and high tissue penetration depth [1–4]. The antitumor effect of SDT is highly dependent on the reactive oxygen species (ROS) caused by acoustic sensitizers and low-intensity ultrasound (US). During the SDT process, tumor cells typically produce high levels of endogenous antioxidant anti-glutathione (GSH) [5–9], attenuating the intracellular ROS level and hindering the efficiency of SDT. To address this issue, researchers often use nanomaterials, such as MnWO₄ [10], MnO₂ [11,12], and Au [13] to achieve GSH depletion [7,14]. The consumption of GSH by these nanomaterials is highly dependent on the levels of intratumoral H₂O₂ and O₂. However, the partial pressure of O₂ inside a tumor is less than 5 mmHg, and the concentration of H₂O₂ is in the range of 10–50 μmol L⁻¹ [15–17]. These tumor microenvironments (TME) are insuffi-

cient for nanomaterials to exert the effective and complete stifling of tumor growth and metastasis, resulting in the weakened efficacy of SDT [4,6,18].

Cancer cells consume glucose through the glycolytic pathway to acquire energy for their growth, a phenomenon known as the “Warburg effect” [19–21]. The rapid proliferation of cancer cells requires an abundant source of nutrients and an adequate energy supply. If cancer cells are unable to obtain a sufficient supply of glucose and other nutrients to maintain their rapid growth, they may die of starvation, which is known as cancer starvation therapy [22–24]. Current starvation therapy is usually dependent on loading glucose oxidase (GOx) into tumors, which can consume glucose and turn it into gluconic acid [25–28]. However, O₂ is essential for this process, so the efficacy of GOx-based starvation therapy is also severely limited by the tumor’s hypoxic environment. Glyceraldehyde-3-phosphoglyceraldehyde dehydrogenase (GAPDH) is a crucial enzyme responsible for promoting the rate of glycolytic processing [29–31]. Therefore, reducing the glycolytic activity of GAPDH in TME would help cut off the energy source allowing cancer growth, thus improving starvation therapy without O₂ participation.

Thiol-ene click reactions are simple and highly efficient reactions between thiols and carbon-carbon double bonds or carbon-carbon triple bonds that are mediated by free radicals [32–34]. Importantly, the reactions do not require the participation of oxygen to consume the thiol substances rapidly [35–39]; this gives it huge potential for depleting the GSH and GAPDH molecules in TME [40], both of which include substantial quantities of thiols. Therefore, the application of the thiol-ene click reaction can overcome the current shortcomings of SDT, which is limited by hypoxia, and cut off the energy supply pathway used by cancer cells to achieve starvation therapy. Unfortunately, thiol-ene click reaction promoting SDT and cancer starvation therapy has not yet been reported as far as we know.

Here, we introduce the thiol-ene click reaction into the treatment of cancer to improve SDT and starvation therapies for cancer. We chose the porphyrin-based two-dimensional (2D) metal-organic frameworks (MOFs) as the ultrasonic sensitizer, which can generate free radicals under US due to the existence of porphyrin molecules [41]. Then, 5-norbornene-2-carbaldehyde (NC) molecules, which can rapidly react with a sulfhydryl group [32,42], were linked onto the 2D Gd-TCPP (TCPP = 4,4',4'',4'''-porphyrin-5,10,15,20-tetratrayltetrabenzoic acid) nanosh-

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eets *via* ligand exchange. A series of *in vitro* experiments confirmed that the synthesized product could trigger thiol-ene click reactions with GSH and GAPDH in cells under US, thus promoting SDT and starvation therapy (Scheme 1). A range of *in vivo* experiments further demonstrated that the product could kill cancer cells with high efficiency and high biosafety. In addition, the product delivered a high relaxation rate of T_1 and was able to undergo magnetic resonance imaging (MRI) in mice *in vivo* due to the involvement of the Gd element, meaning that the product is promising as an MRI diagnosis-guided SDT and starvation therapy with an anticancer effect.

EXPERIMENTAL SECTION

Chemicals

TCPP, *N,N*-dimethyl formamide (DMF), gadolinium(III) nitrate hydrate ($\text{Gd}(\text{NO}_3)_3$), 5-fluoroanthranilic acid (FAA), NC, GSH, ethanol, and ether were purchased from Shanghai Aladdin Chemical Industry. All chemicals were of analytical reagent grade and used without further purification. Ultrapure water was obtained by filtration with a Pincheng system ($R = 18.25 \text{ M}\Omega \text{ cm}$).

Synthesis of Gd-TCPP 2D nanosheets

The 2D MOF Gd-TCPP nanosheets were prepared according to reference [43]. Briefly, 0.03 mmol of TCPP (237.3 mg) was dissolved in 12 mL of DMF, and 0.01 mmol of $\text{Gd}(\text{NO}_3)_3$ (34.4 mg) was dissolved in 1 mL of ultrapure water. The two solutions were then mixed in a Teflon reactor and placed in an oven at 65°C for 10 h. The precipitate obtained was washed with ether several times.

Synthesis of ligand NC-FAA

After dissolving 0.12 mol of NC (14.64 mg) in 20 mL of ethanol in a three-necked flask, 0.1 mol of FAA (15.5 mg) was added. The solution was heated to reflux at 55°C for 40 h. The solvent was removed by rotary evaporation to give brown solid, which was then dissolved with dichloro-methane. The precipitate that could not dissolve was discarded, and the resulting solution

underwent rotary evaporation to remove the solvent, giving brown solid NC-FAA.

Synthesis of Gd-TCPP-NC

In a Teflon reactor, 27.2 mg of Gd-TCPP and 27.5 mg of NC-FAA were dispersed in 12 mL of ethanol and reacted without stirring at 60°C for 12 h. The mixture was centrifuged (8000 r min^{-1}) to collect the precipitate, which was washed several times with ethanol to obtain Gd-TCPP-NC.

Capture of $\cdot\text{OH}$ and alkyl radicals by electron spin-resonance (ESR)

5,5-Dimethyl-1-pyrroline *N*-oxide (DMPO) in $10 \mu\text{L}$ was mixed with $50 \mu\text{L}$ aqueous solution of the material in $100 \mu\text{g mL}^{-1}$ and subjected to US irradiation (1.2 MHz , 1.5 W cm^{-2} , 4 min). The signals of $\cdot\text{OH}$ and alkyl radicals were monitored by an ESR spectrometer.

Detection of GSH and glutathione oxidized (GSSG) in solution

Several aliquots were prepared by mixing GSH ($100 \mu\text{L}$, 10 mmol L^{-1}) with Gd-TCPP-NC nanosheets ($100 \mu\text{L}$, 29 mg mL^{-1}) in $800 \mu\text{L}$ of water, and the supernatant was extracted from respective aliquots at different time points for GSSG and GSH content test by the reductive glutathione assay kit (purchased from the Suzhou Comin Biotechnology Company).

Measurement of relaxivity of MRI

Inductively coupled plasma (ICP) analysis was performed on 10 mL aqueous solutions of Gd-TCPP MOF nanosheets and aqueous solutions of Gd-TCPP-NC MOF nanosheets. The solutions were then diluted to Gd concentrations of 0.01, 0.02, 0.04, 0.08, and 0.1 mmol L^{-1} . The T_1 values of the solutions with different concentrations were obtained with a 0.5 T NIUMAG-MesoMR23-060H-1 at 32°C .

MTT assay for cytotoxicity

Normal human tubular epithelial cells (HKC) and human kidney cancer cells (A498) were inoculated on 96-well plates and

Scheme 1 Schematic diagram of the prepared 2D MOF of Gd-TCPP-NC used for SDT and starvation therapy of tumor *via* thiol-ene click reaction.

incubated with Dulbecco's modified Eagle medium (DMEM, about 1×10^4 cells per well) for 24 h. Then, these cells were incubated with different concentrations of 0–100 $\mu\text{g mL}^{-1}$ of Gd-TCPP or Gd-TCPP-NC nanosheets for another 24 h. 3-[4,5-Dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT 5 g L^{-1} , 10 μL) and dimethyl sulfoxide (100 μL) were added and shaken for 10 min, and the cells were incubated in the dark for 30 min. Each well was measured at 570 nm with an enzyme marker. Cell viability was calculated by comparing the absorbance of treated cells with that of the control. HKC cells and A498 cells were also treated with US for 5 min, and then the absorbance at 570 nm was measured for comparison of cell viability.

Determination of GSH, GAPDH, and pyruvate in cells

After incubation, HKC and A498 cells were collected from the culture dishes by treatment with trypsin and centrifugation, and the contents of GSH, GAPDH, and pyruvate in the cells were determined according to the GSH kit, GAPDH kit, and pyruvate kit, respectively.

Treatment of tumor-bearing mice

All animal experiments were performed according to the guidelines established by the Capital Normal University. The procedures for care and use of animals were approved by the Institutional Animal Care and Use Committee (IACUC) of Capital Normal University. Approximately 2×10^6 cells in phosphate buffer saline (PBS, 100 μL) were injected subcutaneously into the back of each group of six week-old male BALB/c mice (Beijing Charles River Company, License number: SCXK 2016-0006). When the tumor volume reached the desired volume (about 1500 mm^3), *in vivo* experiments were performed.

To assess the therapeutic effect of the material at the animal level, we randomly divided the mice with implanted A498 tumors into three groups ($n = 4$ each group): (i) PBS (10 mL kg^{-1}); (ii) Gd-TCPP (600 $\mu\text{g mL}^{-1}$, 10 mL kg^{-1}); (iii) Gd-TCPP-NC with the same dose as Gd-TCPP. The mice were treated with US every day (1.2 MHz with a power density of 1.5 W cm^{-2} and a duty cycle of 50% for 5 min), for a total of five days [44–47]. PBS, Gd-TCPP or Gd-TCPP-NC was injected *via* the tail vein of the mouse. The tumor size and weight were measured daily. The tumor size was estimated as follows: $V = a \times b^2/2$ (a : the longest tumor diameter; b : the shortest tumor diameter).

MRI imaging of tumor-bearing mice and haematoxylin and eosin (H&E) staining of organs

Homozygous mice injected with Gd-TCPP-NC (600 $\mu\text{g mL}^{-1}$, 10 mL kg^{-1} mice) were anesthetized and imaged with the NIU-MAG-MesoMR23-060H-1 at a predetermined time. On the 21st day after receiving different US treatments, the mice were euthanized. The major organs (liver, spleen, kidney, heart, and lung) of mice were stained with H&E and observed under a microscope.

RESULTS AND DISCUSSION

Gd-TCPP MOF nanosheets were obtained in accordance with instructions from a reference (Figs S1–S6 in the Supplementary information) [43]. NC molecules were first modified with FAA to produce 2-(5-norbornene-2-carbaldehyde)-5-fluorobenzoic acid (NC-FAA), as shown in Fig. 1a (Figs S7 and S8). Then, the

Gd-TCPP nanosheets were linked with NC-FAA by replacing ligand water in Gd-TCPP with the NC molecules, resulting in the final product, which is denoted Gd-TCPP-NC (Fig. 1b, Figs S9 and S10).

Fourier-transform infrared spectroscopy (FTIR) was applied to confirm that the NC group was coordinated with Gd-TCPP. The FTIR spectrum of Gd-TCPP-NC in Fig. 1c (red curve) shows the characteristic peaks of $-\text{C}-\text{N}$ (1089.9 cm^{-1}) and $-\text{N}-\text{H}$ (1606 cm^{-1}) in the TCPP ligands [48–50]. Compared with Gd-TCPP, a peak $\text{C}-\text{F}$ bond at 1049.5 cm^{-1} appeared in the FTIR spectrum of Gd-TCPP-NC [51,52], which came from the NC molecules. This proves that the NC was combined with Gd-TCPP. This was further evidenced by the ultraviolet-visible (UV-vis) absorption spectra (Fig. 1d) and fluorescence spectra (Fig. 1e). They demonstrate that the UV-vis absorption shifted from 421 nm of Gd-TCPP to 416 nm of Gd-TCPP-NC [53–55], and the fluorescence emission at 651 nm of Gd-TCPP shifted to 646 nm of Gd-TCPP-NC [53,56]. Both the blue-shifted UV-vis and fluorescence spectra were due to the F atoms in NC having a stronger electronegativity [54,55]. Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) images (Fig. 1f, g) indicate the 2D nanosheet-like morphology of the prepared Gd-TCPP-NC with plane sizes in several hundred nanometers. Energy dispersive X-ray (EDX) spectroscopy in Fig. 1h demonstrates the Gd, F, N, C, and O elements in the Gd-TCPP-NC with a reasonable stoichiometric ratio.

ESR spectroscopy analysis was employed to investigate the Gd-TCPP-NC's generation of free radicals in the solution under US. We added the radical-trapping additive DMPO to the aqueous solution of Gd-TCPP-NC, which can combine with highly reactive $\cdot\text{OH}$ and alkyl radicals to form stable paramagnetic adducts with ESR characteristics [5]. Fig. 2a shows that the aqueous solutions of Gd-TCPP and Gd-TCPP-NC could both produce $\cdot\text{OH}$ under US. Fig. 2b shows that the aqueous solution of Gd-TCPP could not produce alkyl radicals, while the aqueous solution of Gd-TCPP-NC induced alkyl radicals under US [57].

To confirm that the prepared Gd-TCPP-NC can be used for thiol-ene click reactions with GSH and GAPDH, we tested the depletion of GSH and the inhibition of GAPDH by Gd-TCPP and Gd-TCPP-NC in aqueous solutions under different conditions. As shown in Fig. 2c, in the absence of sonication, the GSH contents of aqueous solutions of both Gd-TCPP and Gd-TCPP-NC remained above 90% for 2 h. Under US, the content of GSH in Gd-TCPP solution dropped to 69%, and the content of GSSG increased to approximately 31% in 2 h (Fig. 2d). In contrast, in the aqueous solution of Gd-TCPP-NC (Fig. 2d), the content of GSH declined to 20% under US, indicating Gd-TCPP-NC consumed much more GSH than Gd-TCPP. The results demonstrate the fact that Gd-TCPP-NC could react with GSH in a thiol-ene click reaction, while Gd-TCPP did not.

Similarly, the activity of GAPDH in Gd-TCPP and Gd-TCPP-NC solutions remained above 94% for 2 h without US (Fig. 2e). Fig. 2f shows that the activity of GAPDH in Gd-TCPP solution was maintained at about 80% under US for 2 h, while the activity of GAPDH in Gd-TCPP-NC solution was only about 40%. This result indicates that Gd-TCPP-NC can rapidly inhibit the activity of GAPDH under US, which is expected to block the energy supply pathway of tumors and achieve effective tumor starvation therapy.

To give insight into whether Gd-TCPP-NC can effectively kill

Figure 1 (a) Diagram showing the synthesis procedure of NC-FAA. (b) Diagram representing the fabrication of Gd-TCPP-NC nanosheets. (c) FTIR spectra of Gd-TCPP, NC-FAA, and Gd-TCPP-NC. (d) UV-vis absorption of Gd-TCPP and Gd-TCPP-NC. (e) Fluorescence signals of Gd-TCPP and Gd-TCPP-NC. TEM image (f), SEM image (g), and EDS spectrum (h) of Gd-TCPP-NC.

cancer cells through thiol-ene click reactions, we monitored the contents of GSH and GAPDH in A498 during the ultrasonic treatment of Gd-TCPP-NC with the control of Gd-TCPP. Here, GSH and GAPDH were analyzed by the kit method after incubation with different contents of Gd-TCPP-NC and Gd-TCPP. At a material concentration of 0, the effect of ultrasound only on the intracellular GSH and GAPDH content was almost negligible, and the relative levels of both were above 95% (Fig. S11). Fig. 3a shows that even at the low strength of $3.125 \mu\text{g mL}^{-1}$ for Gd-TCPP-NC, the relative content of GSH in A498 declined by about 50%, which is far greater than that of 5% for the Gd-TCPP treatment. Furthermore, all the tested GSH contents of Gd-TCPP-NC became much lower than that in Gd-TCPP at the

same level. This demonstrates that the NC group in Gd-TCPP-NC underwent thiol-ene click reactions and consumed large amounts of GSH under US [59,60].

Fig. 3b shows that with an increase in the concentration of Gd-TCPP, the activity of GAPDH declined slightly under US treatment. The decline in the activity of GAPDH was up to 50%, even at a low Gd-TCPP-NC concentration of $3.125 \mu\text{g mL}^{-1}$ (Fig. 3b). The reduction in GAPDH activity was attributed to the activation of the redox switch (cys152) of GAPDH by the alkyl radicals released from Gd-TCPP-NC [58]. At a concentration of $6.25 \mu\text{g mL}^{-1}$ of Gd-TCPP-NC or higher, we found that the activity of GAPDH increased instead (Fig. 3b). This was attributed to the alkyl radicals released from Gd-TCPP-NC at

Figure 2 ESR spectra revealing (a) $\cdot\text{OH}$ and (b) alkyl radicals of Gd-TCPP-NC and Gd-TCPP in water within US (1.2 MHz, 1.5 W cm^{-2} , 4 min, 50% duty cycle). The contents of GSH and GSSG in the supernatant of Gd-TCPP and Gd-TCPP-NC at different time points (c) without US and (d) with US (1.2 MHz, 1.5 W cm^{-2} , 50% duty cycle). Inhibition of GAPDH in the Gd-TCPP and Gd-TCPP-NC solutions (e) without US and (f) with US (1.2 MHz, 1.5 W cm^{-2} , 50% duty cycle).

Figure 3 (a) Relative contents of GSH in A498 cells cultivated with Gd-TCPP and Gd-TCPP-NC under US normalized with that of US alone. (b) Relative contents of GAPDH in A498 cultivated with Gd-TCPP and Gd-TCPP-NC under US normalized with that of US alone. (c) Glycolysis process in carcinomas (modified from Ref. [58]). (d) Relative contents of pyruvic acid in A498 cultivated with Gd-TCPP and Gd-TCPP-NC under US normalized with that of US alone.

high concentrations reacting with GAPDH and forming S-S in cys2991 [58], which further increased the GAPDH activity. Also, the formation of S-S in cys2991 prevented glycolysis in cancer cells (Fig. 3c) [58]. This fact can be reinforced by the decrease of pyruvate (a product of GAPDH glycolysis) in corresponding cancer cells, as shown in Fig. 3d.

To verify the effectiveness of click reactions for killing cancer cells based on the prepared Gd-TCPP-NC consuming GSH and GAPDH in TME, HKC and A498 cells were incubated with the prepared materials. After 24-h incubation with Gd-TCPP and Gd-TCPP-NC without US, the survival rates of both HKC (Fig. 4a) and A498 (Fig. 4b) were above 85%, even at the high material concentration of $50 \mu\text{g mL}^{-1}$ (Fig. 4a, b). This demonstrates that Gd-TCPP and Gd-TCPP-NC had low toxicity to HKC and A498 in the absence of US. In contrast, under US, the survival rate of HKC (Fig. 4c) decreased a little with the increase in the content of Gd-TCPP or Gd-TCPP-NC. The survival rate was kept at approximately 70% at the content of $25 \mu\text{g mL}^{-1}$ for Gd-TCPP or Gd-TCPP-NC. For A498 cancer cells (Fig. 4d), the survival rate decreased more clearly with the incubation of Gd-TCPP-NC under US. It was reduced to approximately 55% at a high concentration of $25 \mu\text{g mL}^{-1}$. This may be due to the different expression levels of GSH and GAPDH in normal cells and cancer cells. Moreover, the survival rate of A498 grown in Gd-TCPP-NC decreased more significantly than that in Gd-TCPP. Even at a low concentration of $3.125 \mu\text{g mL}^{-1}$, the survival rate of A498 grown in Gd-TCPP-NC was only 65%, which is lower than that in $12.5 \mu\text{g mL}^{-1}$ of Gd-TCPP. These results suggest that Gd-TCPP-NC-produced alkyl radicals reacted with sulfhy-

dryl units in the cells by the click reaction under US conditions (Figs S12–S14). Therefore, the killing of cancer cells is more effective by Gd-TCPP-NC than by Gd-TCPP under US.

To evaluate the ultrasonic therapeutic effects of the prepared Gd-TCPP-NC nanosheets *in vivo*, we injected A498 cancer cells subcutaneously into mice to obtain a tumor model. The tumor-bearing mice were randomly divided into three groups ($n = 4$ each group). The three groups of mice were injected once by PBS (pH 7.5), Gd-TCPP solution ($600 \mu\text{g mL}^{-1}$), and Gd-TCPP-NC solution ($600 \mu\text{g mL}^{-1}$) *via* tail vein, respectively, and the injection doses were all 10 mL kg^{-1} . The three groups of mice were then irradiated with US daily (5 min, 1.2 MHz, 1.5 W cm^{-2} , 50% duty cycle) for consecutive 5 days and executed after 21 days. As shown in Fig. 5a, b, as control, the tumors in the mice treated with PBS grew larger under US. However, the tumors volume in the mice treated with Gd-TCPP under US decreased gradually, and the tumor almost disappeared on the 21st day. In contrast, the tumor in the mice treated with Gd-TCPP-NC under US disappeared on the 14th day. These results indicate the Gd-TCPP-NC performed significant inhibition of tumor growth due to the thiol-ene click reactions between Gd-TCPP-NC and GAPDH/GSH in the tumor. After a one-month observation of the treated mice, there was no recurrence of tumor growth, and the mice showed a satisfactory growth status (Fig. S15). Further histological analysis by H&E staining showed no significant damage or inflammation in the tumor tissues treated with Gd-TCPP-NC and negligible side effects on the major organs (Fig. 5c); this further demonstrates the high biosafety of Gd-TCPP-NC.

Figure 4 Cytotoxicity of Gd-TCPP and Gd-TCPP-NC on (a) HKC and (b) A498 without US. Cytotoxicity of Gd-TCPP and Gd-TCPP-NC on (c) HKC and (d) A498 under US.

Figure 5 (a) Representative photos of A498 tumor-bearing mice after SDT treatments for 21 days. (b) Tumor growth curves of each group of mice. (c) H&E staining of major organs (heart, liver, spleen, lung, and kidney) after receiving different US treatments on the 21st day (scale bar: 50 μm).

Figure 6 MRI imaging and relaxivity rates of (a) Gd-TCPP and (b) Gd-TCPP-NC in water. (c) Determinants of slackening rate r^{IS} and r^{OS} . (d) T_1 -weighted MRI images of tumor-bearing mice treated with Gd-TCPP-NC at different times.

In addition, due to the involvement of the Gd element, the prepared Gd-TCPP-NC nanosheets were also measured for potential MRI diagnosis. As a comparison, the Gd-TCPP presented a high r_1 relaxivity of $44.8 \text{ S}^{-1} (\text{mmol L}^{-1})^{-1}$ calculated by

the computational slope from the fitted curve (Fig. 6a), which coincided with the value reported [43]. Interestingly, the r_1 relaxivity of the Gd-TCPP-NC increased to $51.7 \text{ S}^{-1} (\text{mmol L}^{-1})^{-1}$ as seen from Fig. 6b, much higher than those of

commercial contrast agents (Gd-DTPA: $4.52 \text{ S}^{-1} (\text{mmol L}^{-1})^{-1}$) and previously reported Gd-based materials (Table S1) [61,62]. The higher r_1 of Gd-TCPP-NC should be related to the inner-sphere water relaxivity (r_1^{IS}) and outer-sphere water relaxivity (r_1^{OS}), contributing to the restitution ratios per the Solomon-Bloembergen-Morgan (SBM) theory (Fig. 6c) [53,63]. For the r_1^{IS} , the rotational correlation time (τ_R) of Gd-TCPP-NC was longer than that of Gd-TCPP due to the larger molecular weight, inducing stronger r_1^{IS} of Gd-TCPP-NC. For the r_1^{OS} , diffusion correlation time (τ_D) of the extraneous water around Gd-TCPP-NC was longer than that around Gd-TCPP due to the higher hydrophobicity of Gd-TCPP-NC [64–66]. Therefore, the enhanced r_1^{IS} and r_1^{OS} of the Gd-TCPP-NC increased its r_1 relaxivity.

To confirm the potential of Gd-TCPP-NC for *in vivo* MRI, we injected 300 μL of Gd-TCPP-NC solution ($600 \mu\text{g mL}^{-1}$, 10 mL kg^{-1} mice dose) into an A498 tumor-bearing mouse *via* tail vein, which was then monitored in MRI. Fig. 6d displays that during the period from 0 to 6 h, the T_1 imaging of the tumor site gradually became brighter and clearer, indicating that Gd-TCPP-NC entered the tumor tissue due to its tumor-targeting ability. At 6 h, the tumor site of the mouse had the strongest T_1 -weighted MRI signal. The tumor signal gradually weakened during 10–24 h, indicating that the Gd-TCPP-NC was gradually metabolized by the mouse, and signaling good biosafety of the material. The results suggest the prepared Gd-TCPP-NC had favorable potential as a T_1 -weighted MRI contrast agent.

CONCLUSIONS

In summary, we prepared 2D MOF Gd-TCPP nanosheets coordinated with NC *via* ligand exchange. *In vitro* experiments showed that Gd-TCPP-NC can quickly exhaust GSH and suppress the glycolytic activity of GAPDH by undergoing a thiol-ene click reaction without the participation of oxygen, leading to a fall in the GSH level and pyruvate (a product of glycolysis). Treatment experiments on tumor-bearing mice reconfirmed that Gd-TCPP-NC can achieve excellent cancer therapeutic effects by combining SDT with starvation therapy through thiol-ene click reactions to kill tumors. Furthermore, the r_1 relaxation rate of Gd-TCPP-NC was as high as $51.7 \text{ S}^{-1} (\text{mmol L}^{-1})^{-1}$; further MRI application in tumor-bearing mice demonstrated it as an excellent T_1 contrast agent. This work suggests the potential of the thiol-ene click reaction in oncology combination therapy. Furthermore, this is a pioneering study that opens up future therapeutic integrations of antitumor therapies, the design of materials, and biological and medical areas.

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Author contributions Jiang S, Zhang W, and Tian Y designed the experiments. Jiang S performed the experiments and prepared the manuscript. He Q, Ye L, and Zhang W helped analyze some data on biology. Li C and Dang K helped analyze the data of the materials. Tian Y supervised the project and revised the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

Supplementary information Experimental details and supporting data are available in the online version of the paper.

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通过金属有机框架材料产生巯基-烯点击反应促进声动力-饥饿疗法治疗肿瘤

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摘要 缺氧的肿瘤微环境(TME)限制了声动力疗法(SDT)杀灭癌细胞的效果, 而且癌细胞中过量的谷胱甘肽(GSH)进一步加剧了这种疗效限制。同时, 癌症饥饿疗法作为一种有前途的临床治疗手段已得到越来越多的认可, 但基于葡萄糖氧化酶(GOx)的饥饿疗法的疗效也因缺氧问题而受到限制。甘油醛-3-磷酸脱氢酶(GAPDH)是一种关键的糖酵解酶, 可作为缺氧情况下饥饿疗法的靶点。本文提出利用二维金属有机框架(MOF)实现巯基-烯点击反应, 达到对肿瘤进行SDT和饥饿疗法联合治疗的目的。实验结果表明, 因所制备的MOF材料和癌细胞中硫醇物质发生巯基-烯点击反应, 该MOF可以在TME中无氧消耗GSH和GAPDH。进一步的体外和体内实验表明, 所制备的MOF材料可以有效地杀死癌细胞。这项研究有望为巯基-烯点击反应在SDT疗法和癌症饥饿疗法的应用中开辟一条前景广阔的道路。