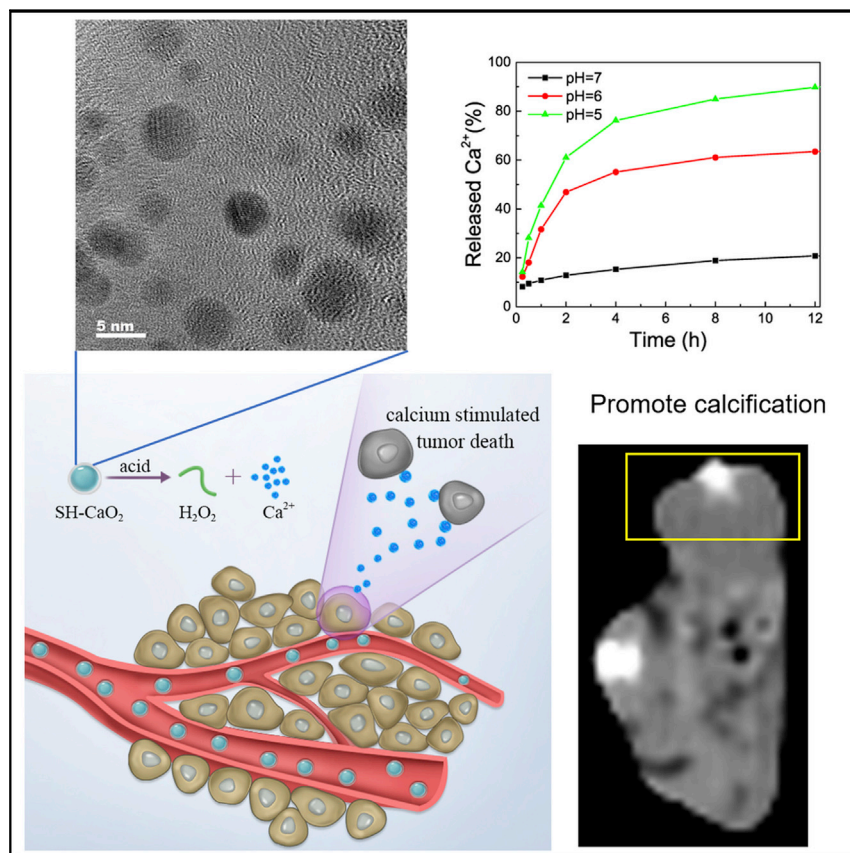


Article

Calcium-Overload-Mediated Tumor Therapy by Calcium Peroxide Nanoparticles



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HIGHLIGHTS

A simple synthetic strategy for ultrasmall calcium peroxide nanoparticles is reported

The nanoparticles show pH-sensitive calcium ions and H_2O_2 release

The release behavior realizes tumor calcium overload and promotes calcification

Great antitumor efficacy is verified for modified calcium peroxide nanoparticles

With their unique biological effects on tumor microenvironment, catabolites of nanoparticles can make a significant difference for tumor suppression. We report a facile synthesis method of ultrasmall calcium peroxide nanoparticles and demonstrate their rapid decomposition in tumor region. This can trigger a destructive calcium overload process in tumor cells, lead to cell death, and further tissue calcification, which also allows for medical imaging.



Article

Calcium-Overload-Mediated Tumor Therapy by Calcium Peroxide Nanoparticles

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SUMMARY

Calcium overload, characterized by an abnormal cytoplasm accumulation of free calcium ions (Ca^{2+}), is a widely recognized cause of damage in numerous cell types and even of cell death. This undesirable destructive process can become a new tool applicable to cancer treatment. Herein, on the basis of the unique biological effect of Ca^{2+} , we demonstrate a highly efficient strategy for tumor therapy by utilizing pH-sensitive sodium-hyaluronate-modified calcium peroxide nanoparticles (SH- CaO_2 NPs). These NPs create an artificial calcium overloading stress in tumor cells, which is responsible for cell death. Notably, the killing effect is not limited to tumor types or hypoxic cells, and normal cells are more tolerant of the adverse influence of NPs than tumor cells. The Ca^{2+} enrichment also increases the likelihood of tumor calcification, which could contribute to *in vivo* tumor inhibition and facilitate medical imaging to monitor the treatment efficacy.

INTRODUCTION

In clinical tumor treatment, internal calcification has frequently been observed after radiotherapy or chemotherapy in certain tumor types.^{1–3} Calcification may be associated with tumor cell death, but the intrinsic cause of cell death remains unknown. It is well known that the common biological effect of chemotherapy drugs and high-energy radiation is the production of a large number of free radicals in tumor cells.^{4–6} Calcification, a slow progression of calcium salt deposition in local tissues initiated by the accumulation of localized calcium ions, is a possible outcome of the calcium overload process.^{7–9} These factors suggest that the process of calcification comprises a series of secondary reactions caused by free-radical-induced intracellular calcium overload. However, this possibility cannot fully explain why tumors that have become calcified over time often show better treatment response.

Given the importance of calcium ions in multiple cellular processes, including proliferation, metabolism, and death,¹⁰ there may be an alternative explanation. While free radicals exert strong anticancer effects mainly by directly attacking double-stranded DNA sequences and causing irreparable proliferation-related damage, free-radical-induced calcium signaling disorders are also considered to contribute to cell death. In other words, the induced calcium overloading process is not an inconsequential or trivial process but could be another efficient “destructive factor” against tumor cells in addition to free radicals. Likewise, calcification is not a mere by-product of cancer treatment but a strong proof and external manifestation of the role of the calcium overload mechanism. Overall, free radicals and calcium overload in cells do not merely share mutual causation but a mutual reinforcement or synergistic effect for tumor therapy. Unfortunately, the cell-damaging functions of Ca^{2+}

The Bigger Picture

Metal ions with diverse cellular biological effects can play more important roles in cell metabolism and proliferation than expected. Any alteration of the metal-content balance could induce a series of intracellular responses, even cell death. Ferroptosis is one of the typical examples that have been applied in tumor treatment. This inspires further studies of other functional metal ions and the development of novel strategies for efficient cancer therapy. We report the design of pH-responsive calcium peroxide nanoparticles and show that the quick release of calcium ions under tumor microenvironment can efficiently induce calcium overload and calcification, which are both damaging against tumor survival. *In vivo* evaluation also reveals the tumor-suppression efficiency by modified nanoparticles. This strategy, which is denoted as ion-interference therapy, highlights the availability of other metal ions in oncology and opens a new door for further clinical cancer treatment.

have been long neglected in designing nanomaterial-based antitumor drugs, and there are almost no reports on the development of calcium-based nanomedicine to induce intracellular calcium overloading for combined treatment.

Herein, by making good use of the unique cytotoxicity mechanism of Ca^{2+} , we prepared calcium peroxide nanoparticles (CaO_2 NPs) to demonstrate the unexplored role of calcium overload in tumor therapy, which is of equal importance to reactive oxygen species (ROS), and further elaborated its great potential in clinical cancer treatment. CaO_2 NPs are pH sensitive and would slowly decompose into free Ca^{2+} and H_2O_2 in the acidic tumoral microenvironment,^{11,12} leading to intracellular calcium overload and oxidative stress, respectively, in cells at the same time. For tumor cells in which catalase (CAT) is downregulated,¹³ the constant challenge from oxidative stress would alter protein functions, resulting in the desensitization of calcium-related channels followed by an uncontrollable cellular accumulation of Ca^{2+} .¹⁴ This biological effect could hinder the accurate transmission of calcium signals and induce cell death.^{10,15} Meanwhile, the enrichment of Ca^{2+} is more likely to engender tumor calcification, which would benefit *in vivo* tumor inhibition and simultaneously facilitate computed tomography (CT) imaging diagnosis of the treatment efficacy.^{31,32} By comparison, healthy cells are more tolerant of the adverse influence of NPs. This could be attributed to the presence of a sufficient amount of CATs that prevent oxidative activation of the cells and thus allow the exogenous Ca^{2+} to be more efficiently pumped out or stored through calcium channels (Figure 1). When stabilized with sodium hyaluronate through surface modification, these CaO_2 NPs are stable in body fluids until the protective layer is degraded by hyaluronidase.^{16,17} Considering the negligible systemic biological toxicity of Ca^{2+} , ease of particle synthesis, and economically available raw materials, we believe that sodium-hyaluronate-modified calcium peroxide nanoparticles (SH- CaO_2 NPs) have potential utilities for clinical applications in cancer therapy. Besides, the strategy of ion-interference tumor therapy may also hold promise as an effective cancer therapeutic tool complementary to traditional clinical tumor treatment.

RESULTS

Characterization of SH- CaO_2 NPs

Sodium-hyaluronate-modified CaO_2 NPs were prepared through a simple and quick wet-chemistry method in a methanol-water system at room temperature. As shown in transmission electron microscopy (TEM) images, the obtained NPs had good crystallinity, with an average particle size smaller than 5 nm (Figures 2A and 2B), which could be regulated by changing the alkalinity of the mixture during preparation. X-ray diffraction (XRD) characterization confirmed that the diffraction peaks of the as-prepared products are well in accord with those of the tetragonal CaO_2 standard card (PDF#03-0865), signifying the successful formation of CaO_2 crystalline NPs (Figure 2C). Effective modification with sodium hyaluronate, which was performed alongside the nucleation and growth of the CaO_2 NPs to confine the grain size, was achieved by the attraction between the negatively charged ions and positively charged nanocrystals.¹⁸ The zeta-potential changes of the NPs verified this supposition, as the zeta potential decreased by approximately 10 mV after the modification (Figure S1), and thermogravimetric analysis (TGA) showed a greater mass loss, which further indicated the efficient modification (Figure S2). In addition, while the hydrodynamic size distribution was narrow, the hydrodynamic diameter seemed to be larger than the one displayed in the TEM images (Figure S3). This discrepancy could be partially explained by the hydrogen bonding networks among the NPs, which is consistent with the appearance of a broad absorption peak at 3,600–3,200 cm^{-1} in the Fourier transform infrared (FTIR) absorption spectrum that

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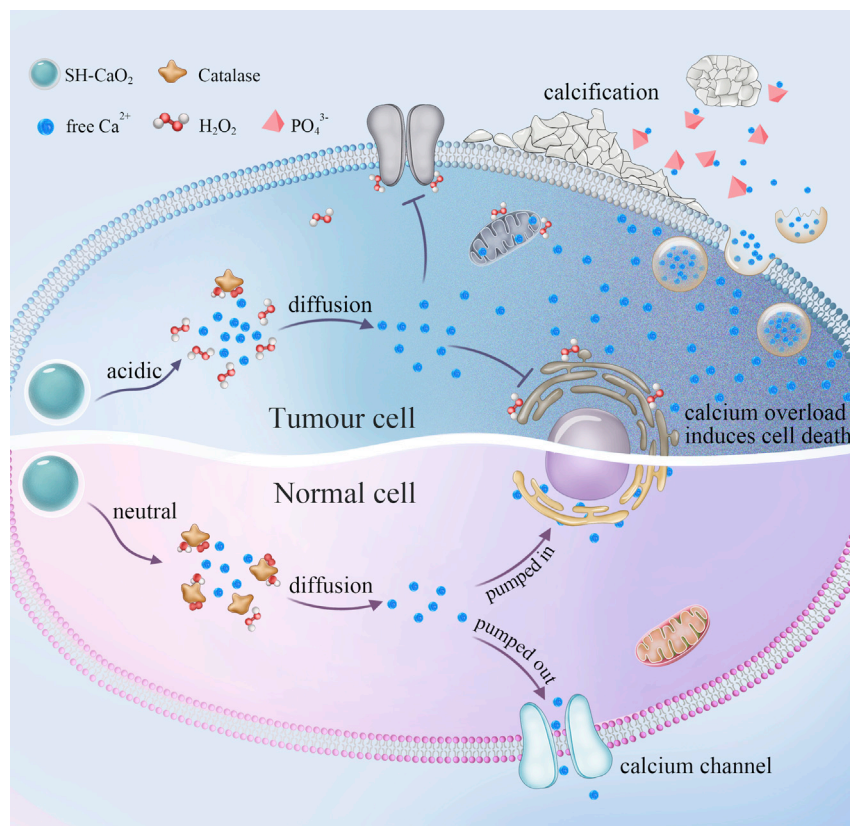
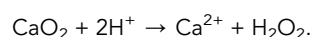


Figure 1. Schematic Representation of the Functional Pattern of SH-CaO₂ NPs

In the acidic microenvironment of a tumor region, SH-CaO₂ NPs are decomposed into Ca²⁺ and H₂O₂ molecules. The low expression of catalase in tumor cells causes abnormal cellular H₂O₂ accumulation and an imbalanced calcium transport pathway, thereby resulting in an efficient cellular calcium overload and subsequently the induction of cell death. Meanwhile, the enriched local concentration of Ca²⁺ increases the likelihood of tumor calcification.

represents the hydrogen-bonded O–H stretching vibration (Figure S4). Moreover, the characteristic peaks around 831, 881, and 1,115 cm^{−1} also verified the existence of peroxide groups.¹⁹

The pH-triggered release of Ca²⁺ by the decomposition of CaO₂ NPs was then evaluated in various pH buffers. Within 12 h, fewer than 10% of the Ca²⁺ were released at pH 7.0, whereas more Ca²⁺ was produced in a mimetic acidic microenvironment, with approximately 50% discharge at pH 6.0 and 80% at pH 5.0 (Figure 2D). This suggested that the CaO₂ NPs were pH sensitive and would decompose into Ca²⁺ in an acidic microenvironment. The reason was unambiguous: in contrast to the slow progression of hydrolysis relying on protons produced by water ionization under neutral conditions, the decomposition speed of CaO₂ NPs would be suddenly accelerated in an acidic environment, depending on the proton concentrations surrounding the NPs. The chemical reaction equation could be written as follows:



Unlike Ca²⁺ already existing in the blood, the leakage of oxidizing H₂O₂ in the blood is not favored. Therefore, sodium hyaluronate as a protective layer was modified on the surface of CaO₂ NPs. The layer could block the direct exposure of the CaO₂ core

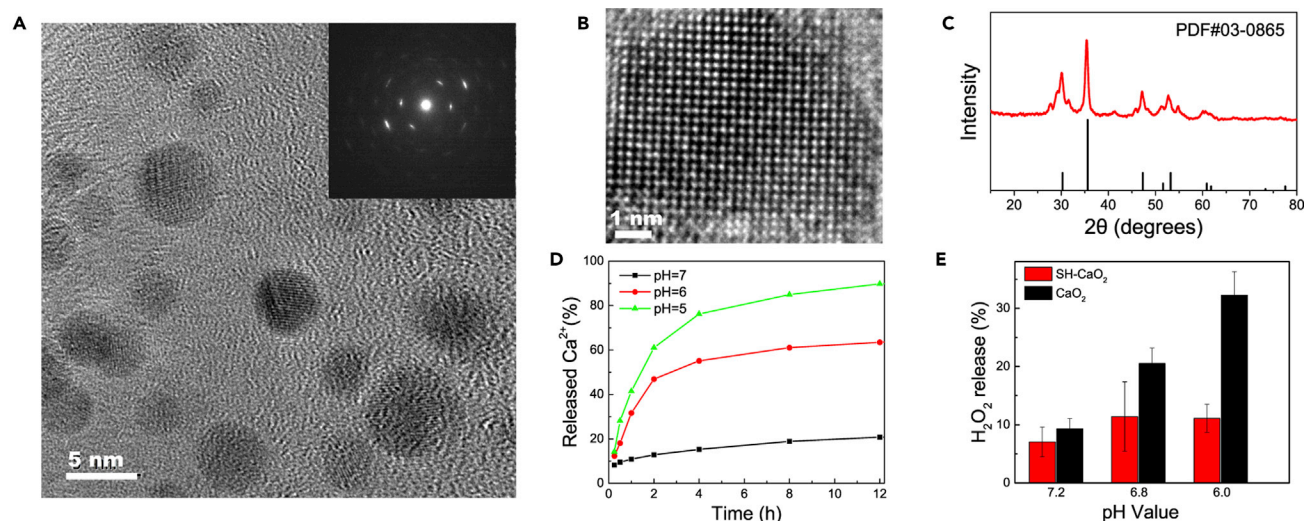


Figure 2. Characterization of SH-CaO₂ NPs

(A) TEM images of calcium peroxide nanoparticles (inset: a TEM electron diffraction image).
 (B) High-resolution TEM image of the calcium peroxide nanocrystals.
 (C) X-ray powder diffraction pattern of the SH-CaO₂ NPs.
 (D) Time-dependent Ca²⁺ release from the CaO₂ NPs dispersed in buffer solutions of different pH values. The release of calcium was clearly accelerated under acidic conditions.
 (E) H₂O₂ production via CaO₂ and SH-CaO₂ NPs over a 2 h timespan at different pH values. The decomposition of NPs was prevented after surface modification with sodium hyaluronate.
 Data are represented as mean ± SD.

to the surrounding protons, thus reducing the degradation of SH-CaO₂ NPs during blood circulation (pH 7.4). Moreover, the -COO⁻ groups in the sodium hyaluronate molecule tended to compete for protons and bind them, which further increased the resistance to some weak pH oscillations. In comparison with unmodified NPs, SH-CaO₂ NPs generated less H₂O₂ under slightly acidic buffer solution, implying that the modified NPs were less harmful before arrival in the tumor region (Figures 2E and S5). Upon uptake by tumor cells, the protective sodium hyaluronate layer of the NPs could be degraded by intracellular hyaluronidase and the CaO₂ core begins to take the therapeutic effect.

In Vitro Cytotoxicity Mechanism of SH-CaO₂ NPs

As a second messenger in intracellular signal transmission, calcium signals are sent through the activation or inactivation of specific targets (proteins, nucleic acids, etc.) to control vital physiological processes, which is achieved essentially under the fluctuations in local calcium concentration.^{20,21} Any Ca²⁺ concentration changes caused by non-cellular regulation would disturb the calcium signals and influence multiple cell activities. In particular, under oxidative stress, the calcium channels in a cell are functioning abnormally, and the cellular Ca²⁺ concentration becomes difficult to re-modulated to a normal state, resulting in calcium-overload-induced cell death.¹⁴ This process can be defined as "calcicoptosis." The hypothesis was firstly evidenced by a simple treatment of Ca²⁺ plus H₂O₂ with 4T1 cells, which showed a sharp decrease in cell viability and an enhancement of intracellular Ca²⁺ concentration (Figure S6). Then, SH-CaO₂ NPs were studied in detail. While incubation with Ca²⁺ alone or a low concentration of pH-sensitive SH-CaO₂ NPs was harmless to 4T1 cells, higher concentrations of NPs demonstrated a rapid increase in cytotoxicity (Figure 3A). This result could be explained by the excessive hydrogen-peroxide-induced oxidative stress, switching the regulatory "Ca²⁺ code" to a signal for cell

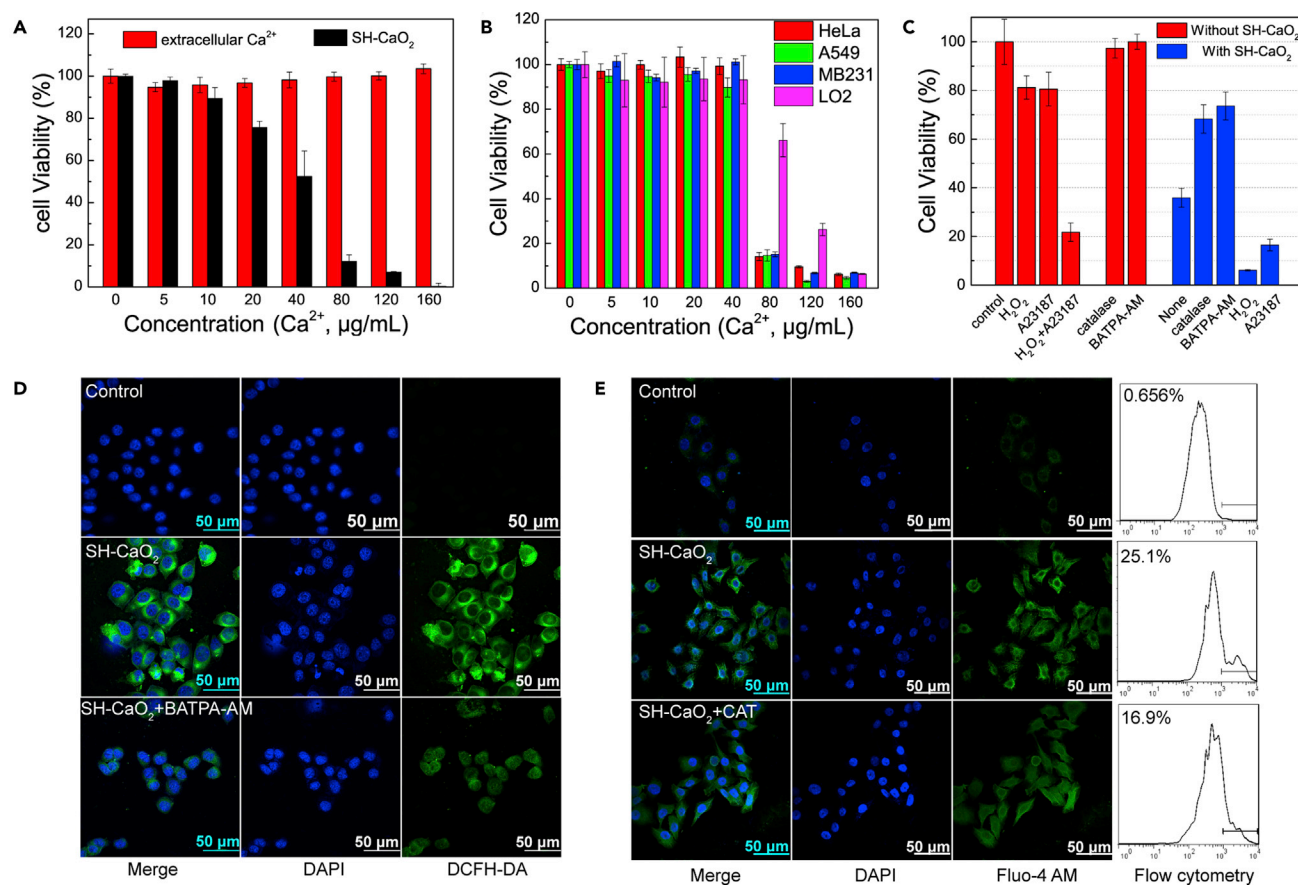


Figure 3. SH- CaO_2 NPs Could Efficiently Induce Tumor Cell Death by Releasing Massive Amounts of Free Calcium and H_2O_2

(A) Cell-viability assay in 4T1 cells incubated with CaCl_2 or SH- CaO_2 NPs.

(B) Cell-viability assay in human tumor cells (HeLa, A549, and MB231) or normal cells (LO2) incubated with SH- CaO_2 NPs. Normal cells were more tolerant of SH- CaO_2 NPs.

(C) Cell-viability assay in 4T1 cells incubated with or without SH- CaO_2 NPs and different reagents, including A23187 (calcium ionophores), BAPTA-AM (calcium chelators), H_2O_2 , and catalase. Ca^{2+} played an essential role in cell death and were of equal importance to H_2O_2 .

(D) ROS production in 4T1 cells after SH- CaO_2 NP treatment.

(E) Free-calcium generation in 4T1 cells after SH- CaO_2 NP treatment.

Data are represented as mean \pm SD.

death. Satisfactorily, the remarkable killing effect was not limited by tumor type, as the SH- CaO_2 NPs could efficiently destroy multiple types of human tumor cells, including cervical, breast, and lung carcinoma, whereas normal human cells managed to tolerate the adverse influence of NPs with much higher cell viabilities than tumor cells under the same treatment. This higher tolerance was attributed to the presence of a sufficient amount of CAT to prevent the normal cells from entering an oxidative stress state caused by excessive H_2O_2 (Figure 3B). Furthermore, SH- CaO_2 NPs were still effective against hypoxic tumor cells, with approximately 80% cell damage when incubated with 1 mmol/L NPs, revealing the potential of treating hypoxic tumors (Figure S7).

To further validate the pivotal role of calcium overload in cell death, we first incubated H_2O_2 and A23187, a calcium ionophore that specifically carries extracellular Ca^{2+} into cells, concurrently with 4T1 cells for 24 h, simulating the working pattern of SH- CaO_2 NPs in cells. In contrast to the 80% cell viabilities after treatment with H_2O_2 or A23187 alone, the cell survival rate of “simulated NPs” dropped sharply

to approximately 22% at the same concentration. Moreover, when the excessive Ca^{2+} ions (or H_2O_2) released by SH- CaO_2 NPs were inhibited by the calcium chelator BAPTA-AM (or CAT), the NP-induced cell damage was reduced by more than half; in contrast, the addition of extra calcium ionophores (or H_2O_2) along with SH- CaO_2 NPs would double the cell death (Figure 3C). All these results illustrated that the role of Ca^{2+} was as important as that of H_2O_2 in the CaO_2 -induced cell death procedure *in vitro*.

The generation of ROS and Ca^{2+} was then monitored by confocal laser scanning microscopy (CLSM). The control group exhibited weak green luminescence, while the group cultivated with SH- CaO_2 NPs exhibited strong intracellular luminescence, indicating the release of the exogenous free Ca^{2+} and H_2O_2 (Figures 3D and 3E). Interestingly, the addition of calcium chelators decreased the H_2O_2 output, as the fluorescence weakened after treatment with NPs and BAPTA-AM. Similarly, the intracellular free- Ca^{2+} output was also reduced by incubation with CAT and NPs (Figure 3E). This phenomenon further indicates the interrelationship between calcium and H_2O_2 in cells.^{20–23} Because both are signaling molecules, the induced increases of calcium overload and ROS will create a self-amplifying loop in cells.^{22,23} On the one hand, the cytosol Ca^{2+} accumulation will stimulate NADPH oxidase activity that is responsible for ROS generation, and the process of Ca^{2+} -promoted ATP synthesis can also induce the respiratory chain electron leakage and ROS levels increase.²⁴ On the other hand, ROS increase can provoke Ca^{2+} entry by modulating the plasma membrane Ca^{2+} channels (e.g., VDCC, TRP, and SOCE), intracellular calcium channels (e.g., RyR and IP_3R), and Ca^{2+} ATPases. Therefore, although the incubated SH- CaO_2 NPs can release Ca^{2+} and H_2O_2 , the detected calcium and ROS fluorescence signal is not just for the SH- CaO_2 NPs degradation but also involves the result of cellular stress responses. Downregulating calcium levels (or ROS levels) can relieve the cellular stress response, and cells can jump out of the self-amplifying loop with self-regulation to a chemical equilibrium. As a result, the collected ROS (or calcium) signals finally decreased.

To further “visualize” the intracellular degradation of SH- CaO_2 NPs, we cultivated cells loaded with fluorescent probes with NPs for 4 h and imaged them every 80 s by CLSM (Video S1). In the first 22 min, in addition to the “calcium sparks” phenomenon,^{25,26} we observed dynamic increases in green and red fluorescence, which was direct proof of the generation of Ca^{2+} and H_2O_2 in the cells (Figures 4A, 4B, and S8). Unexpectedly, the intracellular release of Ca^{2+} coincided with an exocytosis process of excessive calcium, accompanied by the formation of numerous small vesicles outside the cell membrane (Videos S2 and S3). Meanwhile, after incubation with SH- CaO_2 NPs, the cell nucleus became condensed, and the mitochondrial activity was drastically reduced (Figures 4C and S9);²⁷ there were further distinguishing morphological changes of apoptosis in the microstructure, such as the marginalization and densification of chromatin into dense masses, mitochondrial swelling, and the vesicular-like expansion and vacuolization of the endoplasmic reticulum (Figure 4D).²⁸ All these phenomena further indicate that the calcium overload had seriously disturbed the normal proliferation of cells and ultimately resulted in cell death.

Formation of Calcified Nodules by SH- CaO_2 NPs

Accompanied by the cell uptake of SH- CaO_2 NPs, we also observed the formation of small vesicles outside of the cell membrane, which was a very fast process that spanned only a few minutes (Figure 5A). Since the formed small vesicles contained high concentrations of free Ca^{2+} similar to those in matrix vesicles (MVs; extracellular organelles formed by chondrocytes or bone cells that functions in osteogenesis and

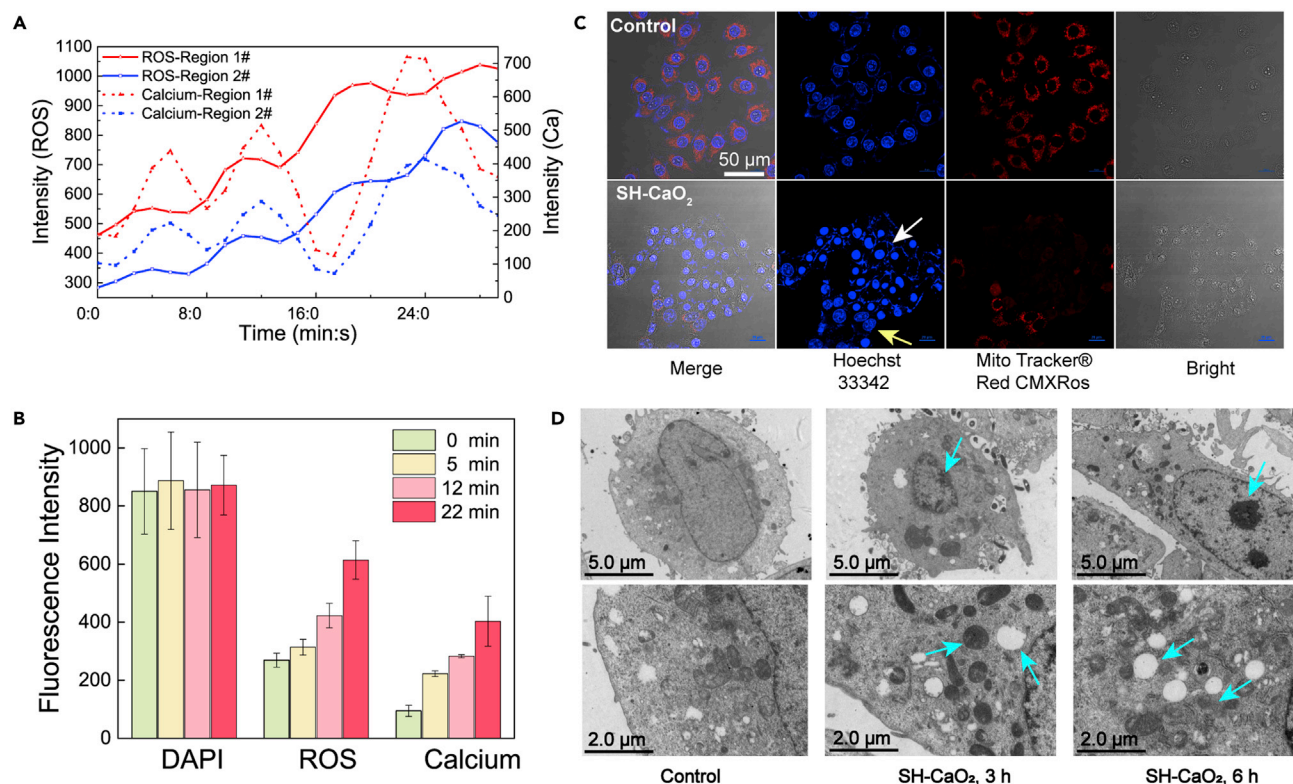


Figure 4. Visualization of the Cellular Release of H₂O₂ and Ca²⁺ and of Cell Death

(A) Time-dependent fluorescence intensity of ROS and calcium in different regions of interest (ROIs) by CLSM (see also Figure S8 and Video S1).

(B) Average fluorescence-intensity changes of ROS and calcium in the marked ROI show intracellular degradation of SH-CaO₂ NPs. Data are represented as mean \pm SD.

(C) Changes of the nucleus and mitochondrial membrane potential before and after the SH-CaO₂ NP treatment as observed by CLSM. The yellow arrow indicates a normal nucleus, whereas the white arrow indicates a condensed nucleus.

(D) Morphological changes in subcellular organelles of tumor cells before and after treatment with SH-CaO₂ NPs for 3 or 6 h (observed by bio-TEM) confirm that the SH-CaO₂-NP-induced calcium overload resulted in tumor death.

mineralization),^{29,30} they were considered to have the same self-calcification ability. Indeed, energy dispersive X-ray (EDX) analysis confirmed the existence of O, Ca, and P elements within the vesicles (Figures 5C, 5D, and S10). Additionally, the collections of cells undergoing exocytosis were also characterized to be the hydroxyapatite phase by XRD, which further validated the calcification potential of the MV-like vesicles (Figure 5B). Biomimetic mineralization was then performed *in vitro* and measured by Alizarin Bordeaux and von Kossa staining, two staining methods that are frequently applied to detect calcified nodules, which are dyed red and black, respectively. In contrast with the control group, there was a wide calcified area in the group incubated with SH-CaO₂ NPs for 6 h; after 12 h of treatment, almost all cells were thoroughly mineralized (Figure 5E). Moreover, this biomimetic mineralization could remain *in vivo* and be imaged by a CT scanner. In the tumor-bearing mouse model, despite the weakly enhanced CT signal 3 h after the injection, SH-CaO₂ NPs could speed up the process of tumor calcification. Three days after a single injection, the tumor region was reduced and brightened in the CT image; for the larger tumor model, the densified microcalcification was more apparent after multiple injections (Figures 6A and 6B). Histological analysis of the tumor sections further illustrated the existence of tumor calcification (Figures 6C and S11). This satisfactory calcification effect would benefit the *in vivo* tumor inhibition and facilitate CT imaging to monitor the efficacy of treatment as well.^{31,32}

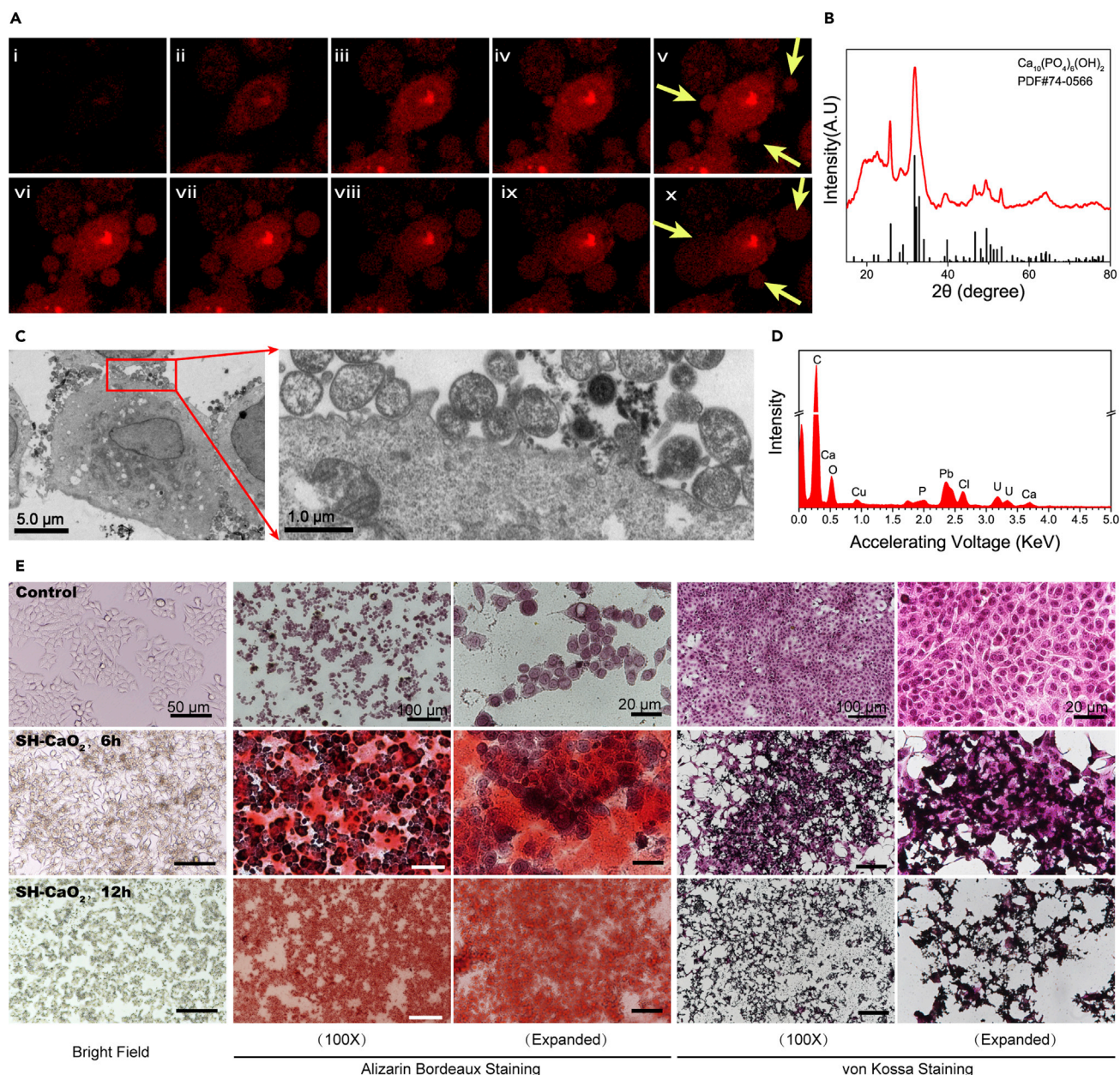


Figure 5. The Observation and Identification of Cell Calcification In Vitro

(A) The formation and exocytosis of calcium-enriched small vesicles after treatment with SH-CaO₂ NPs, as shown in a single cell (yellow arrows). The intracellular free-calcium ions were tracked by a calcium red fluorescent probe (see also [Videos S2 and S3](#)).

(B) X-ray powder diffraction pattern of the collected products from cell exocytosis after treatment with SH-CaO₂ NPs.

(C) Bio-TEM images of 4T1 cells after treatment with SH-CaO₂ NPs for 6 h show the formation of small vesicles outside of the membrane (see also [Figure S10](#)).

(D) Energy-dispersive X-ray (EDX) spectrum of the products of exocytosis after the treatment of SH-CaO₂ NPs shows the existence of mineralization. Cell samples were stained with uranium salts and lead salts for TEM observation.

(E) Identification of the products of exocytosis *in vitro* with Alizarin Bordeaux staining and von Kossa staining, which show the calcified areas in red and black, respectively.

In Vivo Tumor Growth Inhibition by SH-CaO₂ NPs

The antitumor therapeutic effect of SH-CaO₂ NPs was next evaluated in a 4T1 subcutaneous tumor model and an orthotopic tumor model *in vivo*. First, the SH-CaO₂ NPs were proved to be biocompatible and tolerated under the dosage of 75 mg/kg

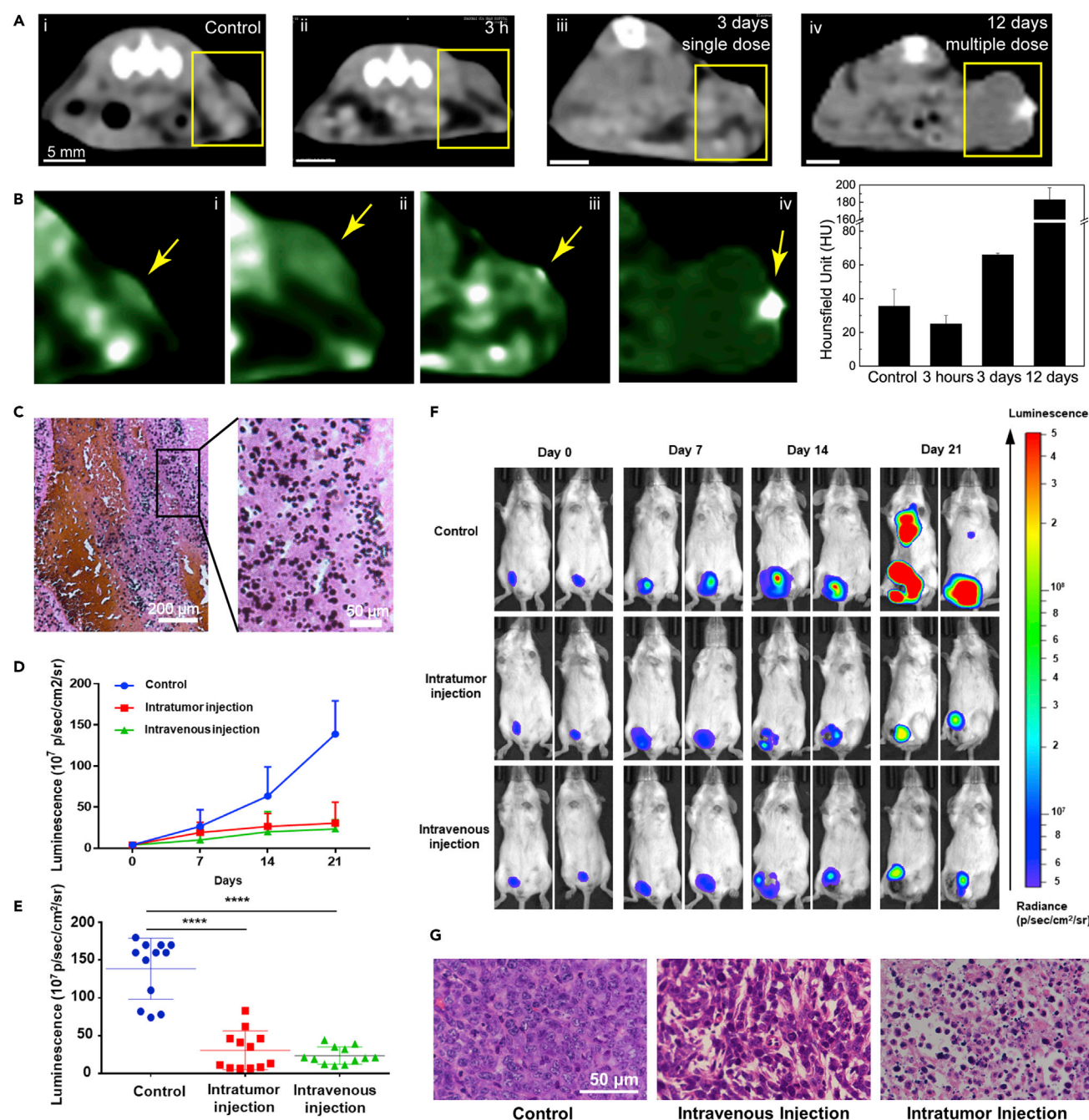


Figure 6. Identification of Calcification in the Tumor Region and Tumor-Growth Inhibition In Vivo

(A) CT images of mice after the following treatments: (i) control, (ii) 3 h after the intratumor injection of SH-CaO₂ NPs, (iii) 3 days after the injection of a single dose of SH-CaO₂ NPs for a small tumor, and (iv) 12 days after the injection of multiple dose (four times, injected every 2 days) for a larger tumor.

(B) Pseudocolor CT images of tumors (i–iv) and the corresponding Hounsfield unit (HU) values of the microcalcification.

(C) von Kossa staining of tumor tissue sections after multiple injections with SH-CaO₂ NPs.

(D) Tumor luminescence changes of different groups after varied treatments (n = 12 in each group).

(E) Tumor luminescence of each mouse in different groups after 21 days of tumor growth.

(F) Photographs of mice tumor luminescence in different groups.

(G) H&E staining of tumor tissue sections after treatment with normal saline or SH-CaO₂ NPs.

Data are represented as mean \pm SD; statistical analysis was performed by Student's t test (****p < 0.0001 versus control).

in vivo given that histological analysis and blood physiological-biochemical analysis revealed no pathological changes in the 30 days following injection (Figures S12 and S13), and the maximum tolerated dose of NPs was then determined to be 110 mg/kg (Table S1; Figures S14–S16). Next, the biodistribution of Ca^{2+} content in major organs suggested the high aggregation and retention ability of SH- CaO_2 NPs in tumor region, ensuring its excellent tumor-killing effect (Figure S17). In subcutaneously implanted tumor models, there was remarkable antitumor efficacy for SH- CaO_2 NPs, such that the tumor almost disappeared during the 14-day observation period after the intratumor injection (Figures S18 and S19). H&E staining and TUNEL (TdT-mediated dUTP nick-end labeling) immunofluorescence also proved cell death 2 days after the injection (Figure S18C). For orthotopic mouse models, in contrast with the control group that have obvious tumor metastasis, both the injection groups exhibited a significant tumor-growth-inhibition effect without distant metastasis (Figures 6D and 6F). The unchanged body weight during the treatments also suggested the great biocompatibility at a given injection dose (Figure S20). Additionally, H&E staining of tumor sections further illustrated the mass death of tumor cells, compared with the controls, while the histological analysis revealed no damages to major organs (Figures 6G and S21). Overall, these therapeutic studies *in vivo* demonstrated that SH- CaO_2 NPs would be a good candidate for effective anticancer therapy with little potential biological side effects.

DISCUSSION

We have reported the room-temperature synthesis of pH-sensitive SH- CaO_2 NPs through a rapid wet-chemistry method and examined the ability of these nanoparticles to release free Ca^{2+} and H_2O_2 in an acidic tumor microenvironment. Because CAT is downregulated in tumor cells, the cells are more vulnerable and easily driven into oxidative stress by excessive cellular H_2O_2 , which may alter the calcium channels and result in abnormal retention of the generated Ca^{2+} in cells. This kind of calcium overload would irreversibly switch the calcium signals from “regulating” to “destroying” and subsequently induce cell death. Since Ca^{2+} are essential in various types of cells, tumor type and oxygen partial pressure do not limit the killing effect. Moreover, SH- CaO_2 NPs are observed to be capable of initiating cell calcification during the therapeutic process, which could further benefit tumor inhibition. Further studies on the specific cell-death pathway of SH- CaO_2 NPs and the mechanism of cell calcification are currently underway. This microenvironment-activated calcification is expected to benefit CT imaging in monitoring the efficacy of treatment.

On a separate note, this calcium-mediated strategy highlights the importance of the biological effects of those metal ions that are often overlooked in conventional tumor treatment, and we summarize this kind of antitumor strategy as “ion-interference therapy.” The *in-situ*-degraded products of nanomaterials—including metal ions, anions, small molecules, and clusters—can really make a difference for tumor treatment with their unique biological effects and interactions with the tumor microenvironment.^{33–36} The emergence of ferroptosis and gas therapy (e.g., NO and H_2S) provides some good examples.³⁷ With the in-depth understanding of their invisible effects to tumor regions and in turn taking advantage of them, the idea of “ion-interference therapy” will potentially open up a new opportunity for the development of antitumor strategies.³⁸

EXPERIMENTAL PROCEDURES

Full experimental procedures are provided in the [Supplemental Information](#).

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at <https://doi.org/10.1016/j.chempr.2019.06.003>.

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AUTHOR CONTRIBUTIONS

Conceptualization, M.Z.; Methodology, M.Z., X.L., and W.B.; Investigation, M.Z., R.S., X.M., Yang Liu, Z.Y., and J.Z.; Writing – Original Draft, M.Z.; Writing – Review & Editing, M.Z., Yanyan Liu, Z.T., Z.Y., X.L., and W.B.; Supervision, X.L. and W.B.; Funding Acquisition, W.B.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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