Research Paper

Cooperation of endogenous and exogenous reactive oxygen species induced by zinc peroxide nanoparticles to enhance oxidative stress-based cancer therapy

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Received: 2019.08.31; Accepted: 2019.09.04; Published: 2019.09.23

Abstract

Reactive oxygen species (ROS)-generating anticancer agents can act through two different mechanisms: (i) elevation of endogenous ROS production in mitochondria, or (ii) formation/delivery of exogenous ROS within cells. However, there is a lack of research on the development of ROS-generating nanosystems that combine endogenous and exogenous ROS to enhance oxidative stress-mediated cancer cell death.

Methods: A ROS-generating agent based on polymer-modified zinc peroxide nanoparticles (ZnO2 NPs) was presented, which simultaneously delivered exogenous H2O2 and Zn2+ capable of amplifying endogenous ROS production for synergistic cancer therapy.

Results: After internalization into tumor cells, ZnO2 NPs underwent decomposition in response to mild acidic pH, resulting in controlled release of H2O2 and Zn2+. Intriguingly, Zn2+ could increase the production of mitochondrial O2·- and H2O2 by inhibiting the electron transport chain, and thus exerted anticancer effect in a synergistic manner with the exogenously released H2O2 to promote cancer cell killing. Furthermore, ZnO2 NPs were doped with manganese via cation exchange, making them an activatable magnetic resonance imaging contrast agent.

Conclusion: This study establishes a ZnO2-based theranostic nanoplatform which achieves enhanced oxidative damage to cancer cells by a two-pronged approach of combining endogenous and exogenous ROS.

Key words: reactive oxygen species, zinc peroxide nanoparticles, pH-responsiveness, magnetic resonance imaging, cancer therapy

Introduction

Reactive oxygen species (ROS) including hydrogen peroxide (H2O2), superoxide anion radical (O2·-), singlet oxygen, and hydroxyl radical can damage lipids, proteins, and DNA, which would lead to cell death when the ROS level exceeds the cellular antioxidant capacity, a condition referred to as oxidative stress [1-6]. Cells constantly generate endogenous ROS as by-products of aerobic metabolism in mitochondria, and maintain redox homeostasis by controlling the balance between ROS formation and scavenging [7]. More importantly, it has been found that cancer cells show elevated mitochondrial ROS production due to oncogenic stimulation and active metabolism [8-10]. Even
though cancer cells enhance antioxidant capacity to counterbalance the overproduction of ROS, their ROS levels are still closer to the toxicity threshold than that of normal cells, making them more susceptible to further ROS formation induced by exogenous agents [11,12]. Therefore, the development of ROS-generating agents that can effectively raise intracellular ROS levels above the toxicity threshold and cause oxidative stress-mediated cell death would be of great interest in cancer therapy.

There are two possible working mechanisms for ROS-generating anticancer agents: (i) enhancement of endogenous ROS production, and (ii) formation/delivery of exogenous ROS. On the one hand, it is well known that mitochondria are the main source of endogenous ROS, and that inhibition of mitochondrial electron transport chain (ETC) can promote the production of O$_2^{-}$ and H$_2$O$_2$ by increasing leakage of electrons to oxygen (O$_2$) at complexes I and III of the ETC [13,14]. Therefore, several ETC inhibitors have been used to kill tumor cells through endogenous ROS-mediated apoptosis [15]. On the other hand, anticancer agents with exogenous ROS-generating ability, such as photodynamic and sonodynamic sensitizers, are attracting more and more attention [16-21]. In particular, stimuli-responsive nanoplatforms have been actively explored for the formation of exogenous ROS in tumor cells [22-28]. However, to the best of our knowledge, there is a paucity of studies regarding the design of ROS-generating nanosystems that combine endogenous and exogenous ROS to potentiate oxidative stress-induced cancer cell death.

Herein, we report a novel ROS-generating agent based on zinc peroxide nanoparticles (ZnO$_2$ NPs), which simultaneously delivers exogenous H$_2$O$_2$ and Zn$^{2+}$ with the capability of intensifying endogenous ROS production for synergistic cancer therapy (Figure 1). The ZnO$_2$ NPs were stable at neutral pH but decomposed to Zn$^{2+}$ and H$_2$O$_2$ at mild acidic pH. In fact, it has been proved that zinc exerts its antitumor effects through inhibition of ETC and consequent elevation of mitochondrial ROS production [29-34]. As compared to conventional zinc ionophores that have been utilized to increase Zn$^{2+}$ accumulation in cancer cells [35-37], controlled Zn$^{2+}$ delivery systems possess the potential for providing desirable therapeutic outcomes with reduced side effects. In addition, although formation of exogenous H$_2$O$_2$ within cells has been recognized as a promising strategy for tumor therapy [38,39], there has been little work on the preparation of H$_2$O$_2$-releasing systems with stimuli-responsive property. With these considerations in mind, we designed and synthesized poly(vinylpyrrolidone) (PVP)-modified ZnO$_2$ NPs as a pH-triggered ROS-generating agent. Upon internalization in tumor cells, ZnO$_2$ NPs would release exogenous H$_2$O$_2$ and Zn$^{2+}$ able to promote endogenous ROS production in mitochondria, which functioned in a synergistic manner to enhance oxidative stress-based cancer cell killing. The vulnerability of tumor cells to additional ROS and the pH-responsiveness of ZnO$_2$ NPs enabled this ZnO$_2$-based ROS-generating anticancer agent to achieve good therapeutic efficacy with low side effects. Moreover, the ZnO$_2$ NPs was successfully doped with paramagnetic manganese (Mn) via a cation-exchange method. The pH-stimulated Mn$^{2+}$ release during the decomposition of Mn-doped ZnO$_2$ (Mn-ZnO$_2$) NPs endowed them with activatable magnetic resonance imaging (MRI) contrast ability, which is useful to monitor the dissociation of ZnO$_2$ NPs as well as the subsequent therapeutic process. This study not only highlights the great potential of ZnO$_2$ nanomaterials in cancer theranostics, but also provides a paradigm for designing stimuli-responsive ROS-generating nanoagents that exacerbate oxidative damage to cancer cells through collaboration between endogenous and exogenous ROS.

Figure 1. Schematic illustration of theranostic ZnO$_2$ NPs for MRI and enhanced oxidative stress-based cancer therapy. Upon endocytosis by tumor cells, ZnO$_2$ NPs undergo dissociation in response to mild acidic pH, causing the release of H$_2$O$_2$ and Zn$^{2+}$. The exogenously released H$_2$O$_2$ and Zn$^{2+}$, that can increase the production of mitochondrial O$_2^{-}$ and H$_2$O$_2$, by inhibiting the electron transport chain (ETC), act synergistically to promote cancer cell killing through the cooperation of endogenous and exogenous ROS. Moreover, Mn-doping via partial cation exchange imparts ZnO$_2$ NPs with pH-activated MRI contrast ability.

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Materials and methods

Materials

Zinc acetate (Zn(OAc)₂, 99.99%), polyvinylpyrrolidone (PVP, Mw = 10000), hydrogen peroxide (H₂O₂, 30 wt. % in H₂O), manganese(II) chloride (MnCl₂, 99%), zinc chloride (ZnCl₂, 99.999%), zinc oxide nanoparticles (ZnO NPs, 20 wt. % in H₂O), zinquin ethyl ester (95%), 3-(N-morpholino)propanesulfonic acid (MOPS, 99.5%), sodium acetate (99%), acetic acid (99.7%), 2',7'-dichlorofluorescin diacetate (DCFH-DA, 97%), thiazolyl blue tetrazolium bromide (MTT, 97.5%), and propidium iodide (PI, 94%) were purchased from Sigma-Aldrich. Apoptosis kit with annexin V-FITC and PI, hydrogen peroxide assay kit, and calcein-AM were obtained from Fisher Scientific.

Synthesis of PVP-modified ZnO₂ NPs

0.1 g of Zn(OAc)₂ and 0.1 g of PVP were dissolved in 5.0 mL of water. Then, 0.5 mL of H₂O₂ was added quickly with vigorous stirring. After reaction for 24 h, the resulting PVP-modified ZnO₂ NPs were washed several times and then re-dispersed in water.

Synthesis of Mn-ZnO₂ NPs

The Mn-doping was achieved by a cation-exchange approach. Briefly, the aqueous solution of ZnO₂ NPs was mixed with the same volume of MnCl₂ with different Mn concentrations. After stirring at room temperature for 4 h, the obtained Mn-ZnO₂ NPs were collected by centrifugation (15000 rpm, 15 min).

Decomposition of ZnO₂ NPs

The release of Zn²⁺ and H₂O₂ from ZnO₂ NPs was determined by dialysis. The aqueous solution of ZnO₂ NPs was dialyzed against acetate buffer (pH 5.5) or MOPS buffer (pH 7.4). The Zn²⁺ was measured by inductively coupled plasma optical emission spectrometry (ICP-OES) and the H₂O₂ was measured by using a hydrogen peroxide assay kit.

Intracellular release of Zn²⁺

Zinquin ethyl ester, a cell-permeable fluorescent probe for Zn²⁺, was used to evaluate intracellular Zn²⁺ release. After incubation with ZnO₂ NPs for 4 h, U87MG cells were stained with 25 μM zinquin ethyl ester at 37 °C for 30 min. Then, the fluorescence images were collected by an Olympus IX81 fluorescence microscope.

Oxidative stress assessment

Intracellular oxidative stress was determined by using DCFH-DA as a probe. U87MG cells seeded in 6-well plates (2×10⁵ cells/well) were exposed to H₂O₂, ZnCl₂, H₂O₂ plus ZnCl₂, or ZnO₂ NPs for 4 h. After staining with 10 μM DCFH-DA at 37 °C for 30 min, the fluorescence images were obtained.

In vitro cancer therapy

To demonstrate the synergistic anticancer effect of Zn²⁺ and H₂O₂, U87MG cells (5×10³ cells/well) were seeded in 96-well plates were incubated with H₂O₂, ZnCl₂, or H₂O₂ plus ZnCl₂ (molar ratio, 1:1) for 24 h. Then, the cell viability was measured by MTT assay. Similarly, the in vitro anticancer activity of PVP-modified ZnO₂ NPs was examined.

Magnetic resonance imaging

To confirm the pH-activated MRI contrast effect, the Mn-ZnO₂ NPs were dispersed in different buffer solutions for 4 h, and then the samples were imaged by an MRI scanner. For in vivo MRI, U87MG tumor-bearing mice were injected with Mn-ZnO₂ NPs (200 μL, [Mn] = 1 mM) through the tail vein. Then, the T₁-weighted MRI images were obtained by an MRI scanner.

In vivo tumor growth inhibition

Mice bearing U87MG tumors (~50 mm³) were injected intravenously with different formulations every other day for four doses, including (1) saline, (2) H₂O₂, (3) ZnCl₂, and (4) ZnO₂ NPs. Each dose of H₂O₂: 0.05 mmol/kg, ZnCl₂: 0.05 mmol/kg, ZnO₂ NPs: 5 mg/kg. The tumor volume and body weight of each mouse were recorded every 2 days.

Results and discussion

The ZnO₂ NPs were fabricated by reacting zinc acetate with H₂O₂ in the presence of PVP at room temperature for 24 h. As shown in the transmission electron microscopy (TEM) image (Figure 2A), the as-prepared ZnO₂ NPs had a uniform particle size of about 50 nm. Dynamic light scattering (DLS) results revealed that the average hydrodynamic diameter of ZnO₂ NPs was ~66.1 nm (Figure 2B). In contrast to zinc oxide nanoparticles (ZnO NPs), ZnO₂ NPs exhibited a strong band at 840 cm⁻¹ in the Raman spectra assigned to O-O stretching vibration (Figure 2C) [40], clearly demonstrating the existence of peroxide ions (O₂²⁻). Moreover, the successful synthesis of ZnO₂ NPs was further confirmed by X-ray photoelectron spectroscopy (XPS). An O 1s peak with binding energy of 532 eV corresponding to O₂²⁻ was observed for the ZnO₂ NPs, whereas the O 1s peak of ZnO NPs was located at 530 eV attributed to O²⁻ (Figure 2D, Figure S1) [41].
To demonstrate the pH-dependent dissociation behavior of ZnO<sub>2</sub> NPs, we measured the release of Zn<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> from ZnO<sub>2</sub> NPs under neutral (pH 7.4) or acidic (pH 5.5) conditions. It can be seen in Figure 3A,B that both Zn<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> were gradually released from the ZnO<sub>2</sub> NPs at pH 5.5. In contrast, the release rates were relatively slow in pH 7.4 buffer solution. As shown by TEM (Figure 3C), ZnO<sub>2</sub> NPs were stable at pH 7.4 but degraded at pH 5.5. Thus, the accelerated release in mild acidic environment can be ascribed to the pH-responsive degradation of ZnO<sub>2</sub> NPs, which is favorable for in vivo tumor treatment since the acidic endo/lysosomes (pH 5.0-6.0) of cancer cells can trigger the release of H<sub>2</sub>O<sub>2</sub> and Zn<sup>2+</sup> on demand. Then, the decomposition of ZnO<sub>2</sub> NPs in cancer cells was confirmed by evaluating the intracellular release of Zn<sup>2+</sup> with a cell-permeable fluorescent Zn<sup>2+</sup> probe, zinquin ethyl ester [42-45]. As expected, ZnO<sub>2</sub> NPs-incubated U87MG cancer cells exhibited stronger blue fluorescence than control untreated cells, and the blue fluorescence increased with increasing concentration of ZnO<sub>2</sub> NPs (Figure 3D, Figure S2), demonstrating the intracellular degradation of ZnO<sub>2</sub> NPs.

To determine whether Zn<sup>2+</sup> and H<sub>2</sub>O<sub>2</sub> could exert anticancer activity in a synergistic manner, we assessed their ability to induce oxidative stress and cytotoxicity. First, the intracellular oxidative stress caused by H<sub>2</sub>O<sub>2</sub> or Zn<sup>2+</sup> was examined by employing 2',7'-dichlorofluorescin diacetate (DCFH-DA) as the probe. After being deacetylated by cellular esterases, DCFH-DA is converted to DCFH that can interact with ROS to generate fluorescent 2',7'-dichlorofluorescein (DCF) [46-51]. As shown in Figure 4A, U87MG cells incubated with either H<sub>2</sub>O<sub>2</sub> or ZnCl<sub>2</sub> displayed significantly greater DCF fluorescence compared with untreated control cells, implying the occurrence of oxidative stress in cancer cells upon H<sub>2</sub>O<sub>2</sub> or Zn<sup>2+</sup> exposure. The Zn<sup>2+</sup>-dependent oxidative stress could be mainly attributed to the increased mitochondrial ROS production [29-34]. Moreover, the DCF fluorescence in U87MG cells co-incubated with H<sub>2</sub>O<sub>2</sub> and ZnCl<sub>2</sub> was higher than that in cells incubated with H<sub>2</sub>O<sub>2</sub> or ZnCl<sub>2</sub> alone, which indicated the combined effect of H<sub>2</sub>O<sub>2</sub> and Zn<sup>2+</sup> on amplification of oxidative stress. The oxidative stress-mediated cytotoxicity of H<sub>2</sub>O<sub>2</sub> and Zn<sup>2+</sup> was further evaluated by MTT assay. As expected, both H<sub>2</sub>O<sub>2</sub> and ZnCl<sub>2</sub> exhibited concentration-dependent toxicity (Figure 4B). More importantly, the viability of U87MG cells co-treated with H<sub>2</sub>O<sub>2</sub> and ZnCl<sub>2</sub> at a molar ratio of 1:1 was lower than that of cells treated with ZnCl<sub>2</sub> or H<sub>2</sub>O<sub>2</sub> alone, demonstrating the synergistic anticancer effect of H<sub>2</sub>O<sub>2</sub> and Zn<sup>2+</sup>. It has been reported that mitochondria, one of the most important organelles...
responsible for energy metabolism, are particularly vulnerable to oxidative damage as they are constantly exposed to high levels of endogenous ROS [52]. Therefore, the synergy between $\text{H}_2\text{O}_2$ and $\text{Zn}^{2+}$ is probably because large amount of exogenously released $\text{H}_2\text{O}_2$ is able to disturb cellular redox homeostasis and then the enhanced mitochondrial ROS production resulting from inhibition of ETC by $\text{Zn}^{2+}$ can lead to the impairment of mitochondria. These results suggested that exogenous $\text{H}_2\text{O}_2$ and $\text{Zn}^{2+}$ with the capability of promoting endogenous ROS production could function cooperatively to enhance oxidative stress-induced tumor cell death.

Next, the in vitro anticancer effect of PVP-modified ZnO$_2$ NPs was examined. As can be seen in Figure 5A, the PVP-modified ZnO$_2$ NPs exerted remarkable therapeutic efficacy against cancer cells, which could be ascribed to the pH-responsive release of $\text{H}_2\text{O}_2$ and $\text{Zn}^{2+}$ from ZnO$_2$ NPs in acidic endo/lysosomes and the subsequent occurrence of oxidative stress (Figure 5B). In addition, ZnO$_2$ NPs exhibited a time- and dose-dependent cytotoxicity (Figure S3). To further confirm ZnO$_2$ NPs-caused cancer cell death, the fluorescent indicators calcein-AM and propidium iodide (PI) were used to stain live (green) and dead (red) cells, respectively. Effective cell killing was observed when U87MG cells were exposed to ZnO$_2$ NPs for 24 h (Figure 5C). The oxidative stress-triggered cancer cell apoptosis was then determined by annexin V-FITC/PI apoptosis detection kit. As shown in flow cytometry profiles (Figure 5D, Figure S4), U87MG cells underwent apoptosis after treatment with ZnO$_2$ NPs for 12 h. Furthermore, the percentage of apoptotic cancer cells increased with increasing dose of ZnO$_2$ NPs. The above results demonstrated the potential of pH-responsive ZnO$_2$ NPs as an efficient therapeutic agent to cause oxidative stress and cancer cell apoptosis.

**Figure 3.** Release profiles of (A) $\text{Zn}^{2+}$ and (B) $\text{H}_2\text{O}_2$ from ZnO$_2$ NPs at different pH values. (C) TEM images of ZnO$_2$ NPs after 2 h of incubation in pH 7.4 (left) and pH 5.5 (right) buffer solutions. (D) Fluorescence images of zinquin ethyl ester-stained U87MG cells after incubation with different concentration of ZnO$_2$ NPs for 4 h. Scale bar: 50 μm.
Theranostic agents that integrate diagnostic and therapeutic functions into a single nanoplatform offer great opportunities for personalized cancer treatment [53-59]. In order to endow ZnO NPs with MRI contrast ability, a facile cation-exchange strategy was employed for the construction of Mn-doped ZnO NPs (Figure 6A). The multifunctional Mn-ZnO NPs were synthesized by mixing ZnO NPs with different amounts of MnCl₂ at room temperature for 4 h. An obvious color change was observed after treatment of ZnO NPs with MnCl₂ (Figure S5). Importantly, there was no distinct morphology difference between ZnO and Mn-ZnO NPs when the weight fraction of Mn was 6.5% or below (Figure S6). The Mn-ZnO NPs with 6.5% of Mn were thus selected for the following studies. Moreover, the energy-dispersive X-ray spectroscopy (EDS) and EDS mapping data also verified the successful fabrication of Mn-ZnO NPs (Figure 6B, Figure S7).

Inspired by the pH-dependent dissolution behavior of ZnO NPs, we compared the longitudinal ($T₁$) relaxivity of Mn-ZnO NPs dispersed in buffer solutions with different pH values. Intriguingly, the $T₁$ relaxation rate ($r₁$) value increased from 0.59 to 4.68 mM⁻¹ s⁻¹ as the pH decreased from 7.4 to 5.5 (Figure 6C, Figure S8), revealing the pH-activated MRI contrast performance of Mn-ZnO NPs. Such an activatable $T₁$ contrast effect is mainly due to the fact that pH-stimulated release of Mn²⁺ from Mn-ZnO NPs provides more efficient chemical exchange between Mn²⁺ ions and protons. The off-to-on contrast ability in response to specific stimuli makes Mn-ZnO NPs highly promising for tumor MRI in vivo. To verify this, U87MG tumor-bearing mice were injected with Mn-ZnO NPs through the tail vein and then imaged by an MRI scanner. As shown in Figure 6D, positive contrast effect was found at the tumor site after intravenous administration of Mn-ZnO NPs. Furthermore, the MRI $T₁$ signal in the tumor region gradually increased with time, which could be attributed to the high tumor uptake of Mn-ZnO NPs. These results indicated that Mn-ZnO NPs is a promising MRI contrast agent for cancer imaging.
Figure 5. (A) In vitro anticancer activity of ZnO$_2$ NPs after 24 h of incubation. (B) DCF fluorescence of U87MG cells after 4 h of incubation with 10 $\mu$g mL$^{-1}$ ZnO$_2$ NPs. Scale bar, 50 $\mu$m. (C) Calcein-AM (green, live cells) and PI (red, dead cells) co-stained fluorescence images of cells treated with different concentrations of ZnO$_2$ NPs for 24 h. Scale bar, 100 $\mu$m. (D) Flow cytometry data showing apoptosis in U87MG cells after exposure to ZnO$_2$ NPs for 12 h.

Figure 6. (A) Scheme showing the preparation of Mn-doped ZnO$_2$ NPs through a facile cation-exchange approach. (B) EDS mapping of Mn-ZnO$_2$ NPs. (C) The $T_1$ values of Mn-ZnO$_2$ NPs under different pH conditions. (D) $T_1$-weighted MRI of U87MG tumor-bearing mice after intravenous injection of Mn-doped ZnO$_2$ NPs. The red circles indicate the tumor area.
In view of the potent anticancer activity of PVP-modified ZnO \(_2\) NPs \textit{in vitro}, we further investigated their feasibility for inhibiting tumor growth \textit{in vivo}. Mice bearing U87MG tumors were treated with different formulations by intravenous injection, and tumor size was measured every other day using a caliper. As can be seen in Figure 7A, the growth of tumors in mice injected with ZnO \(_2\) NPs was significantly suppressed, demonstrating the high therapeutic efficacy of ZnO \(_2\)-based oxidative stress inducers. In comparison, both \(\text{H}_2\text{O}_2\) and \(\text{ZnCl}_2\) showed only limited tumor growth inhibition (Figure S9), most likely due to the rapid clearance after intravenous administration. Moreover, no significant body weight loss was observed after various treatments (Figure 7B,C). Hematoxylin and eosin (H&E)-stained images of major organs collected from different groups of mice at day 14 showed no obvious organ injury (Figure S10). Then, the oxidative stress-stimulated cancer cell apoptosis was determined by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) assay. Tumor tissues derived from mice treated with ZnO \(_2\) NPs had significantly more apoptotic cells as compared to that from saline-injected mice (Figure 7D). In addition, H&E staining indicated that tumors in ZnO \(_2\) NPs-treated mice exhibited severe damage (Figure 7E). The above results suggested that ZnO \(_2\) NPs are good candidates for cancer treatment \textit{in vivo}.

**Conclusions**

In summary, we have successfully developed a ZnO \(_2\) NPs-based ROS-generating nanoagent that enhances oxidative stress-mediated cancer cell killing through the collaboration of endogenous and exogenous ROS, and demonstrated its use in the fabrication of activatable theranostic nanoplatform for MRI-guided tumor treatment. After uptake by cancer cells, the pH-responsive ZnO \(_2\) NPs, in addition to releasing exogenous \(\text{H}_2\text{O}_2\), also provide \(\text{Zn}^{2+}\) to facilitate the production of endogenous \(\text{O}_2\cdot^-\) and \(\text{H}_2\text{O}_2\) from mitochondrial ETC, enabling highly effective synergistic tumor therapy. The pH-dependent dissociation behavior of ZnO \(_2\) NPs, together with the fact that cancer cells are more vulnerable to additional ROS than normal cells, allows them to efficiently trigger cell apoptosis in tumor and show negligible

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**Figure 7.** (A) Tumor growth curves of U87-bearing mice injected intravenously with different formulations. (B) Survival curves of mice in different groups. (C) Body weight changes of mice during the observation period. (D) TUNEL and (E) H&E staining of tumor tissues derived from different groups. Scale bar in d, 50 \(\mu\)m.
damage to major organs. Moreover, manganese could be easily doped into ZnO2 NPs by cation exchange to impart them with pH-activated MRI contrast property, which is appropriate for monitoring the dissolution of ZnO2 NPs and subsequent therapeutic property, which is appropriate for monitoring the dissolution of ZnO2 NPs and subsequent therapeutic application of ZnO2 nanomaterials for theranostic strategy to further improve oxidative stress-based cancer therapy by stimulating cooperation between endogenous and exogenous ROS.

Abbreviations

ROS: Reactive oxygen species; ZnO2 NPs: zinc peroxide nanoparticles; H2O2: hydrogen peroxide; O2-·: superoxide anion radical; ETC: electron transport chain; PVP: poly(vinylpyrrolidone); MOPS: 3-(N-morpholino)propanesulfonic acid; DCFH-DA: 2',7'-dichlorofluorescin diacetate; TEM: transmission electron microscopy; DLS: dynamic light scattering; XPS: X-ray photoelectron spectroscopy; DCFH-DA: 2',7'-dichlorofluorescin diacetate; H&E: hematoxylin and eosin; TUNEL: terminal deoxyxynucleotidyl transferase-mediated dUTP nick-end labeling.

Supplementary Material


Acknowledgements

This work was supported by the National Natural Science Foundation of China (Nos. 81971621, 81671707), Natural Science Foundation of Guangdong Province (No. 2016A030311054), Research Projects of Guangzhou Science Technology and Innovation Commission (No. 201607010201), Research Fund for Lin He’s Academician Workstation of New Medicine and Clinical Translation, Higher Education Colleges and Universities Innovation Strong School Project (Q17024072), and the Intramural Research Program (IRP) of the NIH, NIH.

Competing Interests

The authors have declared that no competing interest exists.

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