Cyclic enhancement of hypoxic microenvironment via an intelligent nanoamplifier for activated NIR-II fluorescence/photoacoustic imaging-guided precise synergistic therapy

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ABSTRACT

Tumor microenvironment (TME)-activated theranostics is a promising strategy to effectively identify small lesions, improve antitumor efficacy, and reduce the risk of undesired side effects. Hypoxia, as a common characteristic of TME, can serve as a preferred site for stimulus-dependent activation; however, tumor-hypoxia levels in various developmental stages exhibit different characteristics, severely limiting the response sensitivity. Herein, a circulating self-reinforcing hypoxic nanoamplifier (CGH NAs) is developed that utilizes a dual-chain reaction process (internal regulation, internal regulation) to achieve precise activation of NIR-II FL/photoacoustic (PA) imaging-guided synergistic therapy. Inspired by the positive correlation of nitroreductase (NTR) with hypoxia, the CGH NAs encapsulate CQ4T and GOx into NTR-sensitive hyaluronic acid-nitroimidazole (HA-NI) shell via a self-assembly approach, enabling aggregation-caused NIR-II FL quenching and tumor-accurate delivery. When CGH NAs efficiently accumulated in the tumor region, the intensive local NTR reduced hydrophobic –NO2 to hydrophilic –NH2, which lead to disassembly of CGH NAs. The released GOx could consume O2 (internal regulation) and glucose to cut off the energy supply, inducing tumor-starvation therapy; generate gluconic acid and H2O2 (oxidative stress). Meanwhile, the released CQ4T promoted rapid recovery of NIR-II FL signals for imaging-guided PDT, which could simultaneously deplete intratumoral O2 (external stimulation). Remarkably, the strengthened tumor-hypoxia levels in turn accelerated the NTR-responsive degradation of the CGH NAs, thereby achieving high-efficiency theranostic.

1. Introduction

As is well-known, the integration of diagnostic and therapeutic functions has successfully helped doctors to monitor the therapeutic effect in real-time and improve the cure rate of disease [1]. But undeniably, most of the conventional theranostic approaches belong to the “always on” mode that exhibit obvious non-specific distribution, resulting in severe systemic toxicity with rather low efficacy [2]. Thus, the on-demand activation of nanotheranostics has achieved a paradigm shift in tumor-specific drug delivery, which emerged as a promising anticancer modality [3]. The breakthrough development in activated nanotheranostics has put forward higher requirements on diagnostic imaging technology. Admittedly, excellent imaging performance for nanotheranostics should possess unprecedented tissue penetration, spatiotemporal resolution, and good biosafety [4]. Fluorescence imaging in the second near-infrared window (NIR-II FL, 1000–1700 nm) has received increasing attention because of its minimal photon scattering, absorption, and negligible tissue auto-fluorescence [5,6]. Remarkably, activatable NIR-II FL nanoprobes that uncage NIR-II FL signals merely in responding to disease-related biomarkers can effectively boost the detection sensitivity and specificity [7,8]. Encouraged by the advantages of activatable NIR-II FL imaging, the NIR-II FL-based activatable nanotheranostics hold a great promise in clinical imaging and guided therapy.

Previous studies have confirmed that abnormal tumor
microenvironmental (TME) factors such as reactive oxygen species (ROS), enzymes, hypoxia, and weakly acidic were widely considered as tumor-related biomarkers [9]. In particular, hypoxia, which is considered as a key microenvironmental feature of solid tumors, resulting from an imbalance between O2 supply and consumption, can induce reductase overexpression in tumor cells [10]. Nitroreductase (NTR), a highly representative hypoxia-related reductase, has been exploited to control the drug delivery in solid tumors, benefiting from being directly proportional to tumor hypoxia degree [11]. Indeed, in the hypoxic tumor microenvironment, the NTR can catalyze the reduction of nitro groups to the corresponding amino groups and derive reducing equivalents from either nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate [12]. Considering the correlation between NTR and O2 levels, efficient NTR-sensitive delivery systems are needed to provide a dramatic response to subtle changes in NTR existing in the TME. Nitroimidazole-based derivatives, as a promising candidate for triggering response in the hypoxic condition, have been used to precisely measure low O2 concentrations in the tumor region [13]. The specific change of the nitroimidazole delivery systems to tumor hypoxia is not a straightforward achievement, and challenges related to sufficient sensitivity of such systems across different tumor hypoxia stages need addressing.

The main existing strategies to improve the NTR-response sensitivity are to increase O2 consumption or modulate O2 production performance in tumor cells. Among many approaches, glucose oxidase (GOx) as an O2-consuming agent has attracted particular interest, which can rapidly deplete glucose and molecular O2 to achieve low-toxicity tumor starvation and create a local hypoxia [14]. Previous NTR-responsive delivery systems using GOx typically employed a hypoxia-enhancing matrix with entrapped or immobilized GOx to exacerbate local hypoxia and amplified NTR-catalyzed release for early antitumor therapy [15]. Recently, our group reported a tumor-specific activated hollow nanosystem (Ag2S-GOx@BHS NYs) for NIR-II FL/PA imaging-guided multistep tumor therapy. We also demonstrated that utilizing the rapid oxygen consumption during GOx-catalyzed glucose oxidation as a trigger could cause depletion of energy supply and amplified TME property [16]. Unfortunately, restricted by the complexity of the TME, the use of intratumoral responses to aggravate the hypoxia level alone is insufficient to achieve satisfactory hypoxia regulation, which seriously hinders its further clinical application. Photodynamic therapy (PDT) is a favorable noninvasive treatment for tumors, which could convert the intracellular O2 into high-toxic singlet oxygen (1O2) to accurately ablate tumor cells upon laser irradiation [17]. The O2 dependence of photodynamic effects in the tumor region that facilitates its use as an externally stimuli-type O2 regulation modality [18].

Herein, We constructed a cyclically enhanced NTR-responsive nanoamplifier (CGH NAs) for precisely activated NIR-II FL/photoacoustic (PA) imaging-guided tumor synergistic therapy via a dual sensitization strategy (Scheme 1). The CGH NAs utilized a self-assembly approach to encapsulate the CQ4T and GOx into hydrophobic 2-nitroimidazole functionalized NTR-sensitive HA, enabling aggregation-caused fluorescence quenching and tumor-precise delivery. Affected by the intensive local NTR in the tumor region, the CGH NAs rapidly decomposed to release the GOx and CQ4T. The intracellular glucose could be oxidized to gluconic acid and H2O2 by releasing GOx to induce starvation and oxidative stress cytotoxicity. This process not only starved the tumors, but also further in situ aggravated the hypoxic levels (internal regulation) to enhance the NTR-response activity in the tumor region effectively. Meanwhile, the released CQ4T promoted rapid recovery of NIR-II FL signaling to “lighting up” the tumor region, ensuring efficient imaging-
The NTR, a common biomarker of hypoxia tumor, can catalyze the hydrophobic Ni groups to hydrophilic 2-aminimidazoles via a single-electron reduction [20], resulting in dissociation of CGH NAs, and subsequent release of CQ4T and GOx (Fig. 2a). Based on the relationship
guided PDT. Remarkably, the process could also deplete intratumoral O2 (external stimulation), which is combined with the GOx to achieve the self-reinforcing NTR-responsive sensitivity. Overall, this CGH NAs provide several advantages: (1) The CGH NAs utilize the “internal regulation and external stimulation” dual-mechanism to amplify the hypoxia response cyclically, realizing a self-promoting degradation process. (2) The enhanced intratumoral hypoxia levels can positively feedback-regulate to achieve precise release of GOx and CQ4T for “turn on” NIR-II FL imaging and oxidative stress/starvation/PDT combination therapy. We believe the CGH NAs could become a promising platform for specific tumor imaging and therapy.

2. Results and discussion

The NTR-sensitive 2-nitroimidazole (NI) functionalized with HA (NI-HA) readily self-assembled into a nanoparticle, encapsulating CQ4T and GOx in the hydrophobic Ni-HA to form a positive feedback nanoamplifier (CQ4T/GOx@NI-HA, CGH NAs). Transmission electron microscopy (TEM) of CGH NAs displayed spherical morphology and uniform size distribution of 138 nm (Fig. 1a). Whereas dynamic light scattering (DLS) analysis indicated a larger average diameter of CGH NAs (189.4 nm) than that of TEM results (Fig. S1, Supporting Information), probably owing to the presence of the hydration layers [19]. Furthermore, the CGH NAs showed excellent stability in different physical solutions (cell medium, PBS, and fetal bovine serum (FBS)), in which no obvious size change was observed for the nanoparticles after 24 h incubation (Fig. S2). Compared with only CQ4T and NI-HA, the UV-vis spectroscopy of CGH NAs (Fig. 1b) demonstrated two characteristic absorption peaks at 330 nm (-NO2) and 780 nm (CQ4T), respectively, indicating that the CQ4T can be effectively encapsulated in the hydrophobic Ni-HA.

One possible mechanism for the nitro group, as everyone knows, is to control the hydrophilicity/hydrophobicity conversion through a NTR-response process. To explore the in vitro response of CGH NAs, the optical and structural properties were examined in the presence of NTR over time. Upon treatment with the NTR, the absorption intensity of CGH NAs in centrifugal precipitates disappeared gradually at 330 nm (Fig. 1c).

Interestingly, the average hydrodynamic size of CGH NAs (Fig. 1d) displayed a gradual downward trend in contrast to NTR-free solution, which gave further evidence for the NTR-responsive degradation behavior of the CGH NAs. Moreover, the disintegration of CGH NAs can also be clearly observed using TEM. The large-sized CGH NAs (137.6 nm) break down into very small particles (12.8 nm) during the reaction with NTR. These results indicated that the NTR could effectively induce the transformation of Ni from hydrophobic to hydrophilic for responsive decomposition of CGH NAs.

Taking advantage of the disassembly process, the NIR-II FL quenching and recovery of CQ4T in CGH NAs were investigated. We first studied the specificity of the CGH NAs by recording their responses to various biological mediators (Fig. 1e). Encouragingly, only in the presence of NTR did the NIR-II FL signal significantly increase. Such emergence of the NIR-II FL recovery should enable its good anti-interference ability to specifically recognize NTR in complex TME. Next, we examined the response characteristics of CGH NAs to different concentrations of NTR in the range of 0–6 μg/mL (Fig. S3, Supporting Information). It should be noted that the NIR-II FL intensity of the CGH NAs displayed an increasing linear trend with NTR concentration, indicating the NTR sensitivity of CGH NAs. As shown in Fig. 1f, the original NIR-II quenching occurred in CGH NAs, which could be ascribed to the aggregation-caused quenching effect of CQ4T, further confirming the successful encapsulation of CQ4T in the hydrophobic core of Ni-HA. After co-incubation with NTR for 6 h, the NIR-II FL of CQ4T in CGH NAs almost completely recovered, whereas the NIR-II FL intensity was basically unchanged without NTR. In summary, the effective decomposition of CGH NAs holds great promise as an excellent theranostic approach.

Fig. 1. a) TEM image and size distribution (inset) of CGH NAs. b) UV-vis absorption spectra of CQ4T, NI-HA, and CGH NAs. c) Time dependent absorption of the centrifugation product of CGH NAs in the presence of NTR. d) Size changes and TEM images of CGH NAs for different time upon addition of NTR. e) NIR-II FL spectra of CGH NAs under different conditions. f) NIR-II fluorescence spectra of CGH NAs in the presence or absence of NTR after 0 h and 6 h incubation, respectively. Inset: photograph of the NTR-activated NIR-II FL signal under 808 nm excitation (1000 LP, 0.3 W, 50 ms).
among NTR level, hypoxia degree, and degradation of CGH NAs, the
dual-chain reaction process of GOx-mediated glucose oxidation and
CQ4T-induced PDT could aggravate the tumor hypoxia microenviron-
ment and amplify the NTR signals. The specific O\textsubscript{2} consumption effec-
tively promotes a virtuous cycle of CGH NAs degradation, constructing a
highly sensitive response system. This positive cycle could be sensitively
turn on the NIR-II FL via the rapid self-enhanced releasing of CQ4T (as
shown in Fig. 1f), which provides an efficient tool with high sensitivity
and specificity for tumor diagnosis.

Under the guidance of NIR-II FL imaging, the highly efficient PDT
generated by CGH NAs is subsequently discussed. The photodynamic
properties were evaluated with 1,3-diphenylisobenzofuran (DPBF) as a
singlet oxygen (\textsuperscript{1}O\textsubscript{2}) indicator. In vitro mimicry of the tumor hypoxic
microenvironment, the absorption intensity of DPBF in the CGH NAs +
NTR group decreased by 0.52-fold after photoirradiation; the DPBF
signal reduction for only the CGH NAs group without any responsive
degradation was 0.26-fold (Fig. 2b and c). These results revealed that the
released CQ4T upon stimulation with NTR can effectively enhance PDT
ability. Remarkably, continuous oxygen-consumption during the PDT
could feed back into the TME to further exacerbate hypoxia (Equation
(1)).

\[
O_{2} + CQ4T \rightarrow 1O_{2}
\]  

(1)

\[
O_{2} + \text{Glucose} \rightarrow \text{Gluconic acid} + H_{2}O_{2}
\]  

(2)

It is well-known that glucose could be effectively consumed via GOx-
catalyzed glucose oxidation reactions, which played a primary role in
providing energy for tumor metabolism as the main intracellular energy
source. Remarkably, the GOx could also be effectively released from the
CGH NAs during the degradation process, which is expected to achieve
an efficient catalytic therapeutic effect (Equation (2)). To validate this,
the ability of CGH NAs-catalyzed oxidation of glucose was investigated,
and incubation with free GOx served as control. First, the enzyme activity of the GOx loaded in CGH NAs was conducted by incubating them with different concentrations of glucose (Fig. 2d). The $\text{H}_2\text{O}_2$ generation increased gradually with the gradient glucose concentration upon the released GOx from the CGH NAs, and their catalytic activity was comparable to free GOx with the same concentration. In contrast, CGH NAs in non-NTR culture conditions showed low $\text{H}_2\text{O}_2$ yields of less than 46%, suggesting that our designed nanoamplifier could significantly reduce the non-specific catalytic behavior of GOx. On the other hand, the CGH NAs, upon stimulated with NTR, consumed large quantities of glucose, demonstrating that the CGH NAs are expected to provide glucose consumption for starving cancer cells. According to the UV-vis results (Fig. 2e), the typical absorbance peak intensity (415 nm) of the CGH + NTR group showed a time-dependent increase. All results revealed that GOx released from the CGH NAs still possesses intact catalytic activity and high sensitivity to glucose. Since GOx could continuously convert glucose into abundant $\text{H}_2\text{O}_2$ and gluconic acid, the pH of the resulting solution increased gradually with the gradient glucose concentration upon the dual-chain reaction process can realize the consumption of dissolved O$_2$ and starves the cells, but also supplies exogenous $\text{H}_2\text{O}_2$ for oxidative stress-induced cell damage. The production of ROS in the 4T1 cellular further proved such a result by utilizing 2,7-dichlorofluorescin diacetate (DCFH-DA) as an indicator (Fig. 3e). Weak fluorescence was seen in the cells added with CGH NAs only, which might be due to the insufficient GOx-concentration released to enhance intracellular ROS levels. For the cells treated with CGH NAs and glucose in the hypoxic environment, a significant increase in the fluorescence intensity relative to other groups, revealing that the CGH NAs + hypoxia + glucose group can provide abundant $\text{H}_2\text{O}_2$ for the severe oxidative stress in cancer cells.

Encouraged by the NTR-specific NIR-II FL activation of CGH NAs, in vitro experiments were conducted on 4T1 cells for fluorescence imaging-guided photodynamic/starvation synergistic therapy. As shown in Fig. 3f, an impressive death of 4T1 cells (8.1%) is observed after co-incubation with CGH NAs and external glucose under laser irradiation. The cytotoxicity of the CGH + glucose + PDT group is significantly higher than that of the CGH NAs monotherapy group containing only PDT or glucose (GOx), indicating that CGH NAs displayed excellent antitumor ability by the dual-chain reaction process of synergistic therapy. Moreover, it has been found that the cellular viability of 4T1 cells is highly dependent on the dosage of CGH in the high-glucose and hypoxic environment (Fig. 5a, Supporting Information). Since CQ4T-mediated PDT and GOx-induced glucose oxidation could exacerbate hypoxia in tumor cells and accelerate the degradation of CGH NAs, the intracellular hypoxia levels after various treatments were studied using a red hypoxia probe (ROS-ID), which would emit fluorescent after degradation by NTR presented in hypoxic cells (Fig. 3g). As expected, with the introduction of glucose and PDT, a gradual increase in red fluorescence was seen in the cells after incubation with CGH NAs; in contrast, the fluorescence of CGH NAs was much weaker in the cells after treatment with only glucose or PDT. Hence, the O$_2$-consuming process could aggravate the intracellular hypoxic level, and provide a suitable environment to achieve the self-enhanced degradation of CGH NAs. Furthermore, the cell apoptosis was also assessed with AO/EB staining. A majority of dead 4T1 cells are observed when treated with either NIR irradiation or external glucose (Fig. 3h). Comparatively, the CGH + glucose + PDT group showed the strongest inhibitory effect on the cell viability, further demonstrating the effective combination therapy.

Motivated by the excellent absorption in the NIR-I window, we first utilized CGH NAs as PA contrast agents to explore their tumor targeting and tissue distribution. Fig. 4a displays the PA intensities and corresponding images from a series of CGH NAs solutions with different concentrations from 12.5 to 200 μg/mL. It revealed that the PA signals were shown to correlate linearly to the concentration of CGH NAs, thus suggesting its potential for quantitative analysis. Compared to the other groups, the PA spectrum of the CGH NAs shows the best contrast and highest signal, indicating that the CGH NAs had strong PA performance (Fig. 4b). In order to track the tumor accumulation behavior of CGH NAs, in vivo PA imaging was investigated at different time points. As shown in Fig. 4c and d, the PA intensity in the tumor region gradually increased and peaked at 12 h after intravenous administration of the CGH NAs, which is mainly owing to the efficient accumulation of the CGH NAs within the tumor through enhanced permeability and retention (EPR) effect. Also, the PA signals of deoxygenated hemoglobin in tumor tissue were detected to analyze the tumor hypoxia levels (Fig. 4d). The PA intensity exhibited a locally increased concentration of deoxygenated hemoglobin, which resulted from GOx-mediated reduced oxygenation status by decreasing the oxygen supply in tumor tissue. More remarkably, the deoxygenated hemoglobin level reached a maximum after 12 h, and persisted at a high level from 12 to 24 h, which fully demonstrated that the CGH NAs are rapidly decomposed and released a large amount of GOx after reaching the tumor tissue to achieve a cyclic enhancement of hypoxic microenvironment.

The degradation process is accompanied by the release of CQ4T, which is expected to achieve NTR-activated NIR-II FL imaging. The 4T1 tumor-bearing mice were selected to evaluate the capability of activated NIR-II FL performance against the tumor region (Fig. 4f). After intravenous injection of the CGH NAs, no NIR-II optical signals were acquired in
the healthy control mice under 660 nm laser excitation (Fig. S5, Supporting Information). Interestingly, the activatable CGH NAs rapidly and specifically fluoresced the tumor region. The NIR-II FL intensity was distinguishable at 6 h post-injection, reaching a maximum at 24 h post-injection, which was steadily observed as a superior targeted tissue to normal tissue signal ratio (SNR = 11.04) (Fig. 4g). The strong NIR-II FL signal increment at the tumor should be attributed to the effective accumulation through enhanced permeability and retention effect of CGH NAs and simultaneous activation. To evaluate the capability of the dual-chain reaction process for consuming dissolved O2 in the tumor region, the 660 nm laser (0.3 W, 20 min) was applied after 12 h of systemic administration with CGH NAs. Intriguingly, the accumulation of activated CGH NAs and the conversion of molecular oxygen in the TME aggravated the tumor hypoxia and displayed a more significant increase in the NIR-II FL intensity compared with that observed in other groups at 24 h post-injection. The further enhancement of fluorescence signals and signal-to-noise ratio (SNR = 12.38) provided strong support for the dual-chain hypoxia-enhancing process of CGH NAs to facilitate rapid diagnosis in vivo. Moreover, ex vivo NIR-II FL imaging of the dissected tumor and major organs obtained from CGH NAs-treated mice at 24 h post-injection showed the tumor could be specifically illuminated and possessed superior NIR-II FL signal compared with normal organs, which was consistent with the trend of fluorescence in vivo (Fig. 4h). The NIR-II FL co-localization images of the tumor tissues (Fig. 4i) reveal that the CGH NAs produced an intense NIR-II FL signals in the tumor region. Taken together, these results validated that the integration of activated NIR-II FL and PA imaging could offer sensitivity for tumor diagnosis and guide precise synergistic therapy.

Encouraged by the in vitro synergistic therapeutic efficacy and high tumor accumulation of CGH NAs, the antitumor performance in vivo was evaluated against 4T1 tumor-bearing mice. Prior to in vivo experiments, the toxicity of CGH NAs was evaluated by hemolysis assay, and results evidenced no obvious swelling, rupture, and coagulation of red blood cells after blending for 3 h (Fig. S6, Supporting Information). All these
results indicate the excellent blood compatibility of the present CGH NAs. When the tumor volumes reached 80–100 mm³, all the mice were randomly divided into five groups and given the following treatments: PBS (I group), CH (II group), CGH (III group), CH + Laser (IV group), and CGH + Laser (V group). For the laser irradiation groups, the tumor regions of tumor-bearing mice were exposed to a 660 nm laser (0.3 W) for 20 min at 24 h post-injection. After the above treatments, the relative tumor volume was monitored every two days for a 14 day-period to assess the antitumor efficacy.

As shown in Fig. 5a, the CH group had a more than the 10.6-fold increase in tumor volume, which was no significant difference from the control (PBS group), indicating that the nanomedicine without GOx displayed no antitumor activity and was non-effect on the treatment. In the absence of laser irradiation, a certain anticancer effect appeared in the CGH group, presumably due to oxidative stress and glucose starvation originating from GOx-mediated intratumoral catalytic oxidation. Comparatively, with laser irradiation, the CH + Laser group showed relatively strong antitumor activity, resulting in effective growth...
Fig. 5. *In vivo* antitumor efficacy of CGH NAs in 4T1 tumor-bearing mice. a) Relative tumor volume of the 4T1-bearing mice in each group (five groups, n = 4) upon different treatments (5 min exposure using an 660 nm laser at 0.3 W after 12 h post injection), recorded every 2 days. b) The digital photographs and c) average weight of excised tumors on day 14. Data are shown as mean ± SD (n = 4). d) Quantitative analysis of ROS production and e) hypoxia evaluation in different groups. (n = 4, mean ± SD) f) Survival rate and g) body weight of the indicated different groups after treatments for 14 days (n = 4, mean ± SD). h) H&E staining of tumors tissue slices collected from different groups. Scale bar = 50 μm.
suppression (the tumor suppression rate ≈84.91%) but still displayed a little tumor regrowth. Importantly, according to the changes in relative tumor volume as a function of time, the tumor growth was nearly completely suppressed in the CGH + Laser group (95.1% growth suppression relative to control), which is considered to be induced by the cooperative cancer therapies of glucose starvation, abundant H2O2 generation, and PDT. A similar tendency was also observed in the digital photographs and weight of tumors after 14 days of treatment (Fig. 5b and c). These results indicated that the dual-chain reaction process of synergistic therapy (V group) led to nearly complete tumor destruction without recurrence, validating the CGH NAs’s outstanding therapeutic outcome. Through combined in vitro experimental and theoretical analysis, the satisfactory antitumor performance of CGH NAs could be attributed to the cyclic enhancement of the hypoxic microenvironment achieved by O2 consumption and ROS-producing ability.

The results of the ROS content and hypoxia degree in the tumor regions were illustrated in Fig. 5d and e, respectively. The hypoxia levels were detected by adopting hypoxia inducible factor (HIF-1a) [22]. Quantitative analysis of ROS production capacity in the different treatment groups indicated a trend of sequential increase in ROS levels with the introduction of GOx and laser (PDT). Besides, the tumor tissue of the CGH + Laser group exhibited the highest level of HIF-1a. These phenomena could be ascribed to the fact that the CQ4T-mediated PDT and GOx-induced glucose oxidation could aggravate the tumor hypoxia level, thus further increasing the dissociation of CGH NAs, and triggering the self-enhanced oxidative stress/starvation/PDT combination therapy. Unsurprisingly, the survival of mice administered with CGH + Laser (V group) was greatly prolonged (Fig. 5f). In addition, it is noted that the body-weight remained stable for all groups (Fig. 5g), verifying the low adverse effects of the CGH NAs in vivo. After the treatments, all the mice were sacrificed, and their tumors and main organs (heart, liver, spleen, lung, and kidney) were dissected for hematoxylin and eosin (H&E) staining to confirm the antitumor effect of the treatments and the biocompatibility of the CGH NAs. As expected, the typical tumor-cell nuclear dissociation was revealed in the CGH + Laser group (Fig. 5h), which was in accordance with the in vivo antitumor results discussed above.

To illustrate the full clinical potential of CGH NAs, the histocompatibility and hemocompatibility of all the treatments were further verified. The results unveiled that there was no observable tissue damage in the histopathologic images of major organs (Fig. S7, Supporting Information). Meanwhile, the hematological and blood biochemical parameters of the mice have no abnormal changes after treatment with CGH NAs for 14 days, demonstrating the excellent histocompatibility and biosafety of the NAs (Tables S1 and S2, Supporting Information). Combining all of the above results, the novel NTR-responsive nanoamplifier CGH with high therapeutic biosafety are promising candidate for precise synergistic tumor therapy.

3. Conclusion

In summary, this work proposes a novel dual sensitization strategy based on a cyclically enhanced NTR-responsive nanoamplifier (CGH NAs), which has successively achieved activated NIR-II FL/PA dual-modality imaging-guided photodynamic starving/oxidative stress therapy. Based on the characteristics of CQ4T aggregation-caused NIR-II FL quenching, the synthesized CGH NAs can realize fluorescent ‘turn off’. Under the tumor hypoxic microenvironment, the overexpressed NTR could reduce the hydrophobic 2-nitroimidazole to hydrophilic 2-aminoimidazole, and the generation of water-soluble groups on HA lead to the self-promoting activation-degradation process of the CGH NAs was achieved via the dual-chain reaction strategy to amplify the hypoxia response cyclically and induce cancer-cell apoptosis. The CGH NAs with high therapeutic efficacy along with the desirable biosafety may represent a promising intelligent nanomedicine in precision cancer theranostics.

Credit author statement

Ziliang Zheng: Conceptualization: Ideas; formulation or evolution of overarching research goals and aims. Xuejiao Chen: Methodology: Development or design of methodology; creation of models. Rong Dai: Writing - Original Draft: Preparation, creation and/or presentation of the published work, specifically writing the initial draft (including substantive translation). Shutong Wu: Writing Editing: Preparation, creation and/or presentation of the published work by those from the original research group, specifically critical review, commentary or revision – including pre-or postpublication stages. Weiwai Kang: Resources: Provision of study materials, reagents, materials, patients, laboratory samples, animals, instrumentation, computing resources, or other analysis tools. YuFei Qin: Investigation: Conducting a research and investigation process, specifically performing the experiments, or data/evidence collection. Shilei Ren: Data Curation: Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later reuse. Ruiping Zhang: Funding acquisition: Acquisition of the financial support for the project leading to this publication. Zhen Cheng: Supervision: Oversight and leadership responsibility for the research activity planning and execution.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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