

Tumor-selective degradation of PARP-1 enabled by the codelivery of β -lapachone and PROTAC for non-small-cell lung cancer therapy

Shuchan Zheng^{1,§}, Chen Liu^{1,§}, Weicon Chen¹, Na Li¹, Maolin Jiang², Ihsan Ullah³, Zimeng Li¹, Youyong Yuan^{2,3,4}  , and Xinchun Li¹  

¹Department of Radiology, The First Affiliated Hospital of Guangzhou Medical University, Guangzhou Medical University, Guangzhou 510120, China

²School of Biomedical Sciences and Engineering, Guangzhou International Campus, South China University of Technology, Guangzhou 511442, China

³National Engineering Research Center for Tissue Restoration and Reconstruction, South China University of Technology, Guangzhou 510006, China

⁴Key Laboratory of Biomedical Engineering of Guangdong Province, South China University of Technology, Guangzhou 510006, China

[§]Shuchan Zheng and Chen Liu contributed equally to this work.

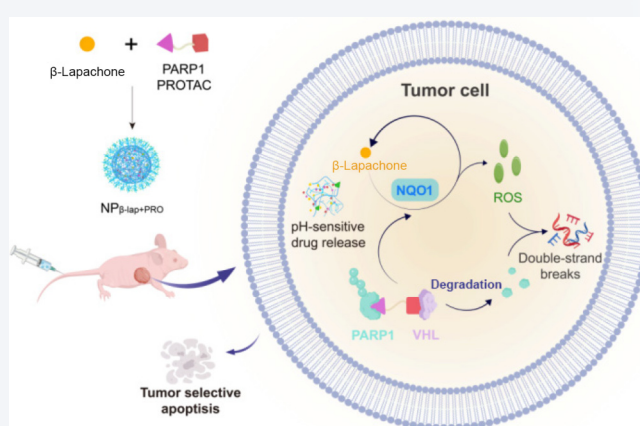
 Cite this article: Nano Research, 2026, 19, 94908191. <https://doi.org/10.26599/NR.2025.94908191>

ABSTRACT: In non-small cell lung cancer (NSCLC), poly(ADP-ribose) polymerase 1 (PARP1) induces genomic instability and promotes tumor progression by impairing DNA repair pathways. Although PARP1-targeting proteolysis-targeting chimeras (PROTACs) offer a promising strategy for selective protein degradation, their clinical application remains limited by poor water solubility and insufficient tumor selectivity. Here, we report a pH-responsive magnetic nanoparticle system co-delivering β -lapachone (β -lap) and a PARP1-targeted PROTAC (PRO) for synergistic and tumor-targeting therapy. Designed with a hydrophobic self-assembled core and a magnetic coating, the nanoparticle (NP _{β -lap+PRO}) enables pH-responsive drug release and magnetic resonance imaging (MRI) monitoring. β -Lap is a bioactivated drug that relies on NAD(P)H:quinone oxidoreductase 1 (NQO1), which is overexpressed in NSCLC cells. It has the potential to deliver tumor-selective DNA damage and induce cell death. The NP _{β -lap+PRO} exploits elevated NQO1 levels in NSCLC to initiate β -lap-driven oxidative stress and DNA damage, while simultaneously enhancing PROTAC-mediated PARP1 degradation within the acidic tumor microenvironment synergistically induces apoptosis. In A549 NSCLC tumor models, this system effectively induces PARP1 degradation, blocks DNA repair, and preserves NAD(P)H pools, thereby amplifying β -lap-induced reactive oxygen species production, leading to enhanced DNA double-strand breaks and apoptosis. This study presents a biomarker-driven nanotherapeutic strategy that integrates PROTAC technology with redox-targeted combination therapy, offering a promising approach for precision treatment of NSCLC.

KEYWORDS: poly(ADP-ribose) polymerase 1 (PARP1), proteolysis-targeting chimeras (PROTACs), β -lapachone, drug delivery, non-small cell lung cancer (NSCLC) treatment

1 Introduction

Globally, lung cancer ranks among the most common and lethal



malignancies [1], with non-small cell lung cancer (NSCLC) comprising approximately 85%–90% of all diagnosed cases [2]. NSCLC is characterized by pronounced genomic instability and recurrent genomic aberrations [3], often driven by impaired DNA repair mechanisms that contribute to disease progression and adverse clinical course [4, 5]. Among the DNA damage signaling networks, poly(adenosine diphosphate (ADP)-ribose) polymerase 1 (PARP1) is a core component [6]. It is an essential enzyme that precisely tunes several DNA repair paths, like base excision repair

Received: August 27, 2025; Revised: October 22, 2025

Accepted: October 23, 2025

 Address correspondence to Xinchun Li, xinchunli@163.com; Youyong Yuan, yuanyy@scut.edu.cn

and the mending of both single-strand DNA breaks (SSBs) and double-strand DNA breaks (DSBs) [7, 8]. PARP1 is frequently overexpressed in NSCLC tumors relative to adjacent normal tissue, and its heightened activity has been associated with disease progression and adverse clinical outcomes, underscoring its potential as a therapeutic target [9–11]. Even though olaparib and talazoparib, serving as PARP inhibitors (PARPis), are effective and have got Food and Drug Administration (FDA) approval for BRCA-mutated breast and ovarian cancers [12, 13], their clinical utility in NSCLC is still limited [14]. Furthermore, conventional PARPis face significant clinical challenges, including drug resistance and off-target toxicities, underscoring the need for alternative treatment strategies [15].

Proteolysis-targeting chimeras (PROTACs) represent a promising strategy in oncology, which achieve the selective degradation of disease-related proteins through the ubiquitin-proteasome degradation system [16–18]. This approach offers distinct advantages over conventional inhibitors, including irreversible target elimination and recyclability [19–21], and the potential to circumvent the resistance arising from PARP1 mutations [22]. However, their clinical translation is hindered by poor tumor selectivity, leading to the off-target effects and toxicity [23, 24]. Notably, β -lapachone (β -lap, clinical code ARQ761) [25] exploits an NAD(P)H:quinone oxidoreductase 1 (NQO1)-dependent redox cycling mechanism [26, 27], enabling the synergistic use via the disruption of DNA repair pathways or selective cytotoxic agents [28]. NQO1 is a cytoplasmic oxidoreductase that catalyzes the two-electron reduction of quinones. It exhibits significant overexpression in numerous human solid tumors, such as breast and lung cancers, compared to normal tissues [27]. High sensitivity to NQO1-overexpressing cells has been observed with the β -lap treatment. Upon the administration of β -lap, NQO1 triggers its futile cycling, during which the compound continuously interconverts among its hydroquinone, semiquinone, and quinone forms. This process progressively depletes cellular NAD(P)H levels and results in the generation of DNA-damaging hydroxyl radicals. Studies show that in NQO1-overexpressing cells, the pretreatment with a PARPi to block the DNA repair, followed by the β -lap treatment, exacerbates the production of reactive oxygen species (ROS) and DNA lesions [29]. β -Lap has demonstrated single-agent anticancer activity in phases I and II of clinical trials [30, 31]. NSCLC exhibits a unique therapeutic vulnerability due to the common co-overexpression of NQO1 and PARP1, making it particularly amenable to targeted therapy [32, 33]. However, this approach faces several limitations, including inherent drug drawbacks, lack of tumor selectivity, and the need for sequential administration. To address these limitations, nanoparticle (NP)-based delivery systems are being developed to improve the drug permeability and enhance the tumor selectivity [34–36], thereby increasing the therapeutic efficacy while minimizing the systemic toxicity [37].

NPs, fabricated from natural or synthetic polymers [38], offer versatile platform for targeted drug delivery [39, 40] with capabilities for the organ or cell-targeted delivery and controlled stimulus responsive drug release [41]. In this study, we developed a pH-responsive [42] nanoplatform (NP _{β -lap+PRO}) that self-assembles into dual-drug-loaded nanoparticles for tumor-targeted co-delivery of β -lap and a PARP1-targeting PROTAC (PRO). Through systematic optimization of the drug ratio, we achieved synergistic inhibition of PARP1-mediated DNA repair and potentiation of

NQO1-dependent ROS generation, both contributing to enhanced cancer cell apoptosis in a tumor-selective manner. The nanoparticle core is formed via self-assembly of poly(lactic acid glycolic acid) copolymer (PLGA) polymers in aqueous solution [43, 44], efficiently encapsulating both hydrophobic therapeutic agents. The surface modification with a Gd-tannic acid (TA) coordination shell enhances the nanoplatform stability, prolongs the systemic circulation, and enables the magnetic resonance imaging (MRI)-based tracking (Fig. 1(a)). When encountering the acidic tumor microenvironment, the nanoparticles undergo dissociation, releasing their payload to initiate a dual mechanism, where the ROS accumulation and DNA damage were amplified via NQO1-dependent β -lap bioactivation and PARP1 degradation, driving cancer cell apoptosis (Fig. 1(b)). The dual-drug-loaded nanoparticles showed marked efficacy in inhibiting tumor growth in an *in vivo* A549 NSCLC model. Crucially, NQO1 activity was essential for the therapeutic synergy, identifying it as a key biomarker for predicting patient response to this treatment strategy. This work establishes a rational design strategy for leveraging nanoparticle-based delivery systems to improve the precision and efficacy of PROTAC-mediated protein degradation in cancer therapy.

2 Results and discussion

2.1 PRO potentiates the therapeutic efficacy of β -lap in A549 NSCLC cells

Studies have shown that PARP1 is activated in response to DNA damage and accelerates ADP-ribose unit polymerization [45], facilitating the rapid aggregation of repair effectors at DNA damage locations, thereby enhancing DNA repair and causing chemotherapy resistance [46, 47]. Targeted inhibition or degradation of PARP1 has been demonstrated to effectively suppress the cancer cell proliferation [48]. In this study, we developed a potent PARP1-targeting PROTAC by conjugating an olaparib-derived PARP1 ligand to a von hippel-lindau (VHL) E3 ligase ligand through an optimized linker (Scheme 1 in the Electronic Supplementary Material (ESM)). The synthesized PROTAC was comprehensively characterized by ¹H nuclear magnetic resonance (NMR) spectroscopy (Fig. S1 in the ESM). The western blot analysis (Fig. 2(a)) confirms the potent and concentration-dependent degradation of PARP1 in A549 NSCLC cells, with a calculated degradation concentration 50% (DC₅₀) of $1.86 \pm 0.53 \mu\text{M}$, demonstrating effective target clearance. The effects of β -lap and PRO on A549 cell viability were measured using the methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay (Fig. 2(b)), whereas the systematic combination screening across multiple dose ratios revealed significantly enhanced antitumor efficacy (Fig. 2(c)). Notably, the combination therapy demonstrated superior *in vitro* cytotoxic efficacy, with combination index (CI) values consistently below 1 (Fig. 2(d)), indicating a synergistic activity against A549 cells. The analysis showed that the β -lap:PRO molar ratio of 1:5 exhibited the more pronounced synergistic effect in A549 NSCLC cells, inducing significant cell death.

Based on these findings, we quantitatively analyzed the changes of poly(ADP-ribose) (PAR) levels in the dual-drug synergy mechanism following the PRO-mediated PARP1 degradation (Fig. 2(e)). The excessive activation of PARP1 during DNA damage catalyzes the synthesis of excess PAR polymers. In A549 cells, sublethal β -lap (2 μM) alone induced the PAR generation, which

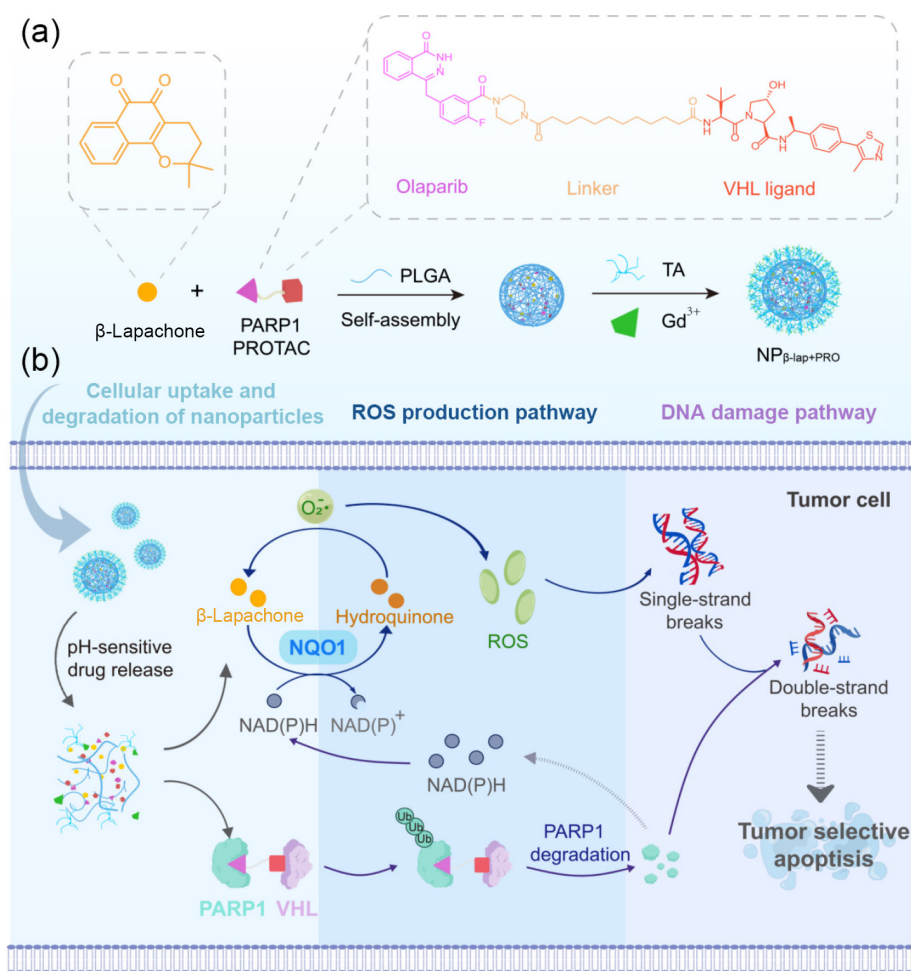


Figure 1 Schematic illustration of the self-assembled nanoparticle NP_{β-lap+PRO} for synergistic cancer therapy. (a) Step-by-step schematic for preparing β-lap and PRO co-loaded nanoparticles. (b) NP_{β-lap+PRO} degrades tumor-specific metabolic proteins for synergistic treatment. Within the acidic tumor microenvironment, NP_{β-lap+PRO} undergoes disassembly, releasing PRO to degrade PARP1. This degradation amplifies the oxidative stress and DNA impairment resulting from β-lap, thereby enhancing therapeutic efficacy.

was exacerbated by the lethal-dose treatment (5 μM). Notably, the co-administration of β-lap (2 μM) and PRO (10 μM) markedly suppressed the PAR accumulation. The pharmacological inhibition of dicumarol (DIC) on NQO1 eliminates the PAR increase induced by β-lap, indicating an NQO1-dependent pathway. Consistent with this, the β-lap monotherapy induced a dose-dependent depletion of the nicotinamide adenine dinucleotide (NAD⁺)/nicotinamide adenine dinucleotide (hydrogen) (NADH) pool reflecting the PARP1's utilization of NAD⁺ as a substrate for ADP-ribosylation of DNA repair proteins (Fig. 2(f)). The PRO-mediated degradation of PARP1 preserved cellular NAD⁺/NADH levels. Moreover, the β-lap induced adenosine triphosphate (ATP) depletion was markedly inhibited by the PRO co-treatment (Fig. 2(g)). This preservation of cellular energy metabolism represents a key basis for their effective synergy, enabling the induction of cancer cell apoptosis rather than simple necrosis. Collectively these results demonstrate that the β-lap-triggered PARP1 hyperactivation drives the PAR synthesis, which is suppressed upon the PRO-mediated PARP1 degradation. By competitively inhibiting NQO1, the DIC pretreatment prevented the catalytic reduction of β-lap. This disruption of β-lap activation undermined the essential requirements for dual-drug synergy. Consequently, the dual-drug treatment prevented excessive PAR accumulation and conserved NAD⁺/NADH and ATP levels,

underscoring the critical role of NQO1 in mediating drug synergy and the mechanism of PRO via targeted PARP1 degradation. To clarify the cell death mechanism, we further examine the roles of β-lap and PRO combination treatment on A549 cells. The results showed the dual-drug treatment triggered canonical caspase-3-dependent apoptosis, an effect blocked by the pan-caspase inhibitor benzyloxycarbonyl-val-alu-asp(ome)-fluoromethylketone (zVAD-fmk) (Fig. 2(h)).

2.2 PARP1 degradation enhances β-lap-mediated oxidative DNA damage and inhibits A549 cell proliferation

Previous studies have established a mechanistic link between β-lap and ROS-mediated DNA damage. Building on this, we investigated the impact of PROTAC-induced PARP1 degradation on β-lap-induced ROS production and DNA damage. Comet assays revealed that the combination treatment markedly increased nucleoid fragmentation, as evidenced by the pronounced comet tail formation (Fig. 2(i)). The quantitative analysis demonstrated significant increases in DNA tail percentage relative to controls (Fig. 2(j)). Specifically, control cells exhibited 15.3% ± 2.7%, whereas the β-lap (2 μM) and β-lap (2 μM) + PRO (10 μM) groups exhibited 41.1% ± 6.1% and 83.9% ± 3.4% tail DNA, respectively,

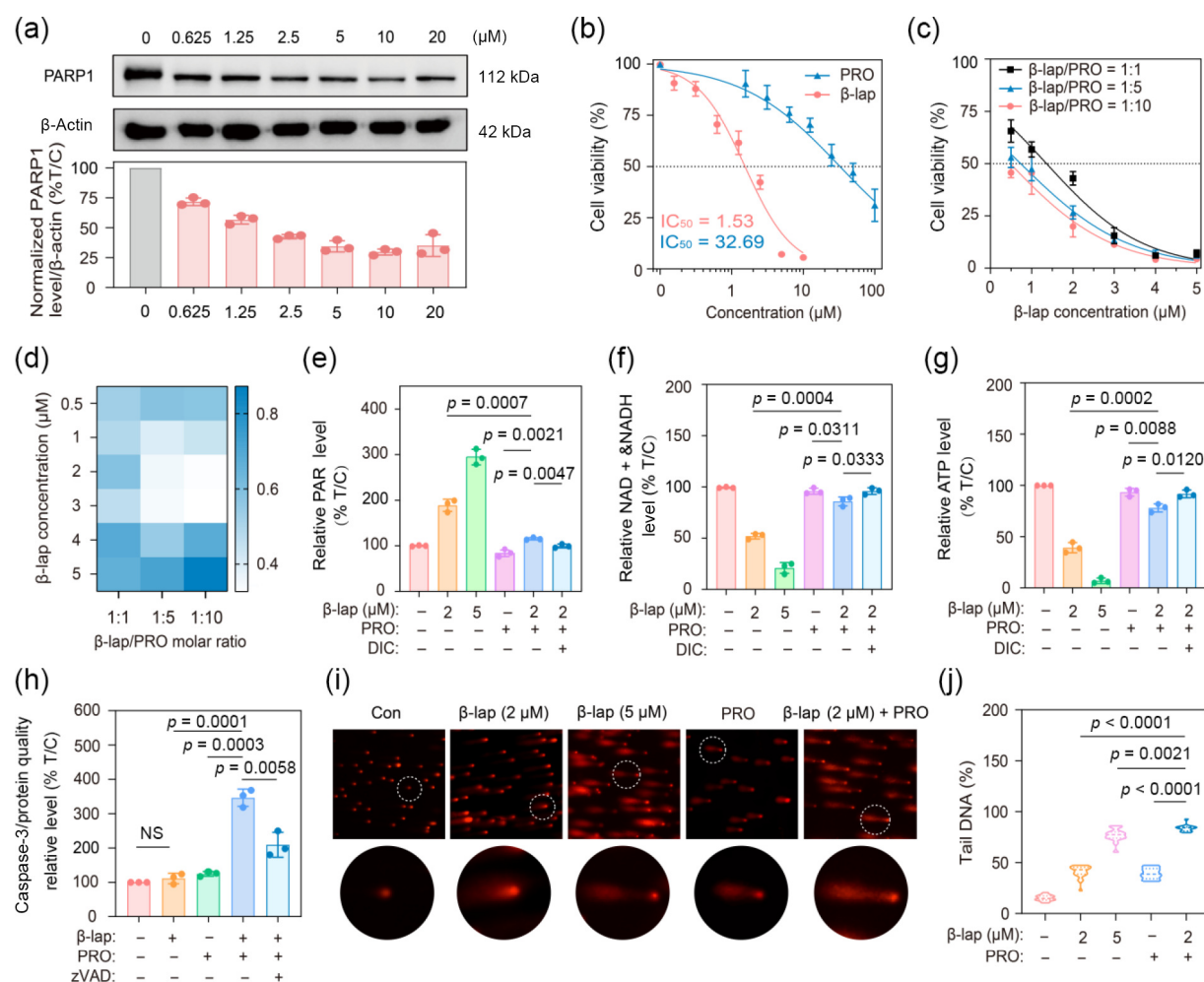


Figure 2 *In vitro* evaluation and intracellular responses of cytotoxicity mediated by β -lap and PRO. (a) Western blot analysis of PARP1 degradation in A549 cells following 48 h treatment with increasing concentrations of PRO; band intensities were quantified using Image J Studio. (b) A549 cell viability was measured via MTT assay following 48 h treatment with β -lap and PRO. (c) The survival capacity of A549 cells under β -lap/PRO treatment in different molar proportions for 48 h was determined by MTT method. (d) CI analysis of different proportions of β -lap and PRO synergistic administration. (e)–(g) PAR, NAD⁺/NADH, and ATP levels in A549 cells treated with β -lap and PRO for 4 h (pretreatment with or without DIC for 2 h). (h) Caspase-3 activation in A549 cells subjected to different drug treatments. (i) and (j) After treating A549 cells with different drug, DNA damage was analyzed and quantified by alkaline comet method. Data are presented as mean $\pm$ SD ($n = 3$).

indicating substantially enhanced DNA damage upon the combination therapy. To further evaluate the nature of DNA lesions, we assessed the DSB formation via the immunofluorescence detection of γ -H2AX[48], a well-established DSB marker. Notably, the dual-drug treatment elicited robust γ -H2AX accumulation (Figs. 3(a) and 3(b)), signifying the synergistic promotion of DSBs. This effect was significantly abrogated by the pretreatment with the NQO1 inhibitor DIC, confirming the NQO1-dependent origin of DNA damage. Collectively, these findings demonstrate that the PARP1 degradation impairs DNA repair processes, thereby converting β -lap-induced primary DNA lesions into lethal DSBs.

β -Lap exerts its anticancer effects primarily through the induction of ROS [49], producing DNA oxidation lesions. In this study, we assessed the ROS generation in A549 cells using the 2,7-dichlorofluorescein diacetate (DCFH-DA) probe. Notably, the combination of β -lap with PRO elicited significantly elevated levels than lethal-dose β -lap alone (Figs. 3(c) and 3(d)). This effect was markedly attenuated by the NQO1 inhibition, confirming the NQO1-dependent ROS amplification. Consistent with the ROS-induced DNA damage pathology, colony formation assays demonstrated significantly enhanced growth inhibitory efficacy of

the combination therapy (Figs. 3(e) and 3(f)). Critically, this synergistic anti-proliferative effect exhibited clear NQO1 dependence, reinforcing NQO1's role as a predictive biomarker for therapeutic response. β -Lap undergoes NQO1-dependent bioactivation, generating the oxidative stress that induces DNA damage and subsequent PARP1 hyperactivation. This triggers excessive PAR formation and NAD⁺/ATP depletion. Our findings reveal that the PRO-induced PARP1 degradation delivers dual benefits, conserving NAD(P)H pools to boost ROS while converting β -lap-triggered SSBs into cytotoxic DSBs via compromised DNA repair. This metabolic shift redirects the cell death from necrosis toward caspase-dependent apoptosis, revealing a combinatorial approach that exploits both oxidative vulnerability and DNA repair deficiency.

2.3 Preparation and characterization of the PLGA hydrophobic core and magnetic shell

We have demonstrated that both β -lap and PRO significantly inhibit the cancer cell growth by triggering apoptosis. However, their clinical application is still hindered by poor water solubility

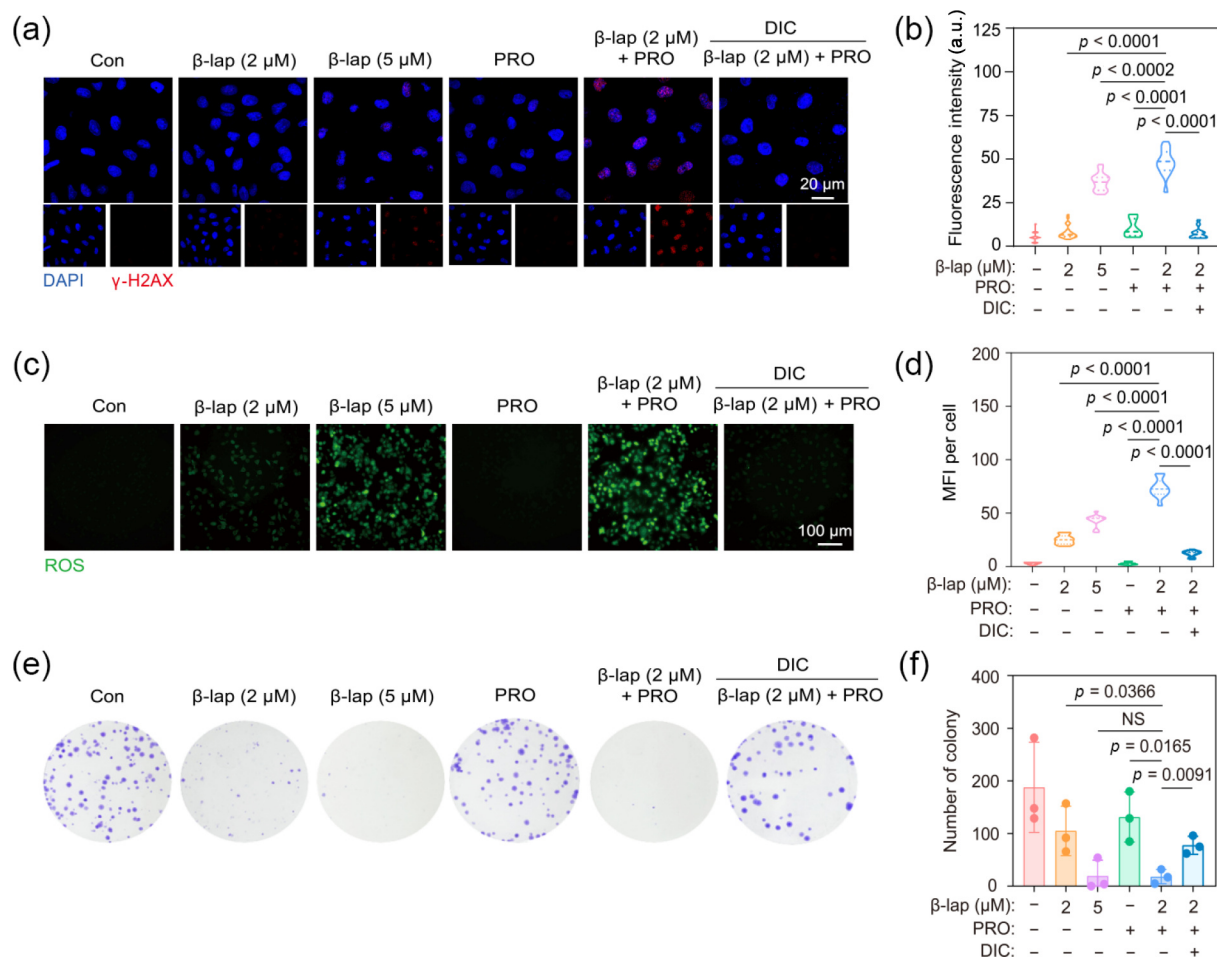


Figure 3 Cellular responses to β -lap, PRO, and combination treatment in A549 cells. (a) and (b) A549 cells were treated with different doses of β -lap, PRO, or their combinations, with or without 2 h DIC pretreatment, using representative confocal images and γ -H2AX immunofluorescence staining quantification. (c) and (d) Fluorescence images of DCFH-DA-detected ROS (green) in treated A549 cells and corresponding mean fluorescence intensity (MFI) quantification. (e) and (f) Treating A549 cells with different formulations, observing colony formation, and quantifying colony numbers. The data are represented as mean $\pm$ SD ($n = 3$).

and non-specific biological distribution. To overcome these limitations, we developed the pH-responsive nanodelivery platform through a stepwise synthesis process (Fig. S2 in the ESM). Among various delivery vectors, PLGA-based polymers demonstrate superior biocompatibility and enhanced drug-loading efficiency [50]. In this study, PLGA nanoparticles were prepared by traditional oil in water single solvent evaporation method, and their physicochemical properties were characterized. Subsequent dynamic light scattering (DLS) showed a hydrodynamic diameter of 85.3 ± 4.5 nm (Fig. S3 in the ESM). The addition of Gd^{3+} and TA initiated spontaneous coordination, led to the formation of a Gd-TA metal-phenolic network. This complex assembled into a hydrophilic metallic shell encapsulating the PLGA core, thereby enhancing colloidal stability (Fig. S3 in the ESM). The hydrodynamic diameter increased to 100.7 ± 2.1 nm (Fig. S3 in the ESM). This Gd-TA shell not only conferred colloidal stability but also introduced functionality for potential imaging or stimuli-responsive behavior. Notably, the final PLGA@Gd-TA formulation maintained an average diameter of ~ 101 nm well within the optimal size range for enhanced tumor penetration and cellular uptake [51], underscoring its promise as an efficient vehicle for targeted anticancer drug delivery.

Comprehensive stability evaluations demonstrated that the nanoparticles maintained robust colloidal stability in normal saline

(NS), deionized water, and 10% fetal bovine serum (FBS) for up to 72 h (Fig. S4 in the ESM), with consistent size distribution profiles (Fig. S5 in the ESM). Notably, cancer cells exhibited a preferential uptake of rigid nanoparticles relative to their softer analogues, in line with previous observations [52]. To interrogate this phenomenon, PLGA-shell nanoparticles were employed as a reference. The quantitative flow cytometry revealed that PLGA@Gd-TA nanoparticles exhibited significantly higher intracellular accumulation compared to PLGA cores at 2, 4, and 6 h post-incubation (Figs. S6(a) and S6(b) in the ESM), substantiating the enhanced cellular affinity of mechanically stiff nanostructures. Collectively, the system synergizes size-optimized biodistribution [53], serum stability, and cellular uptake attributes positionable for precision oncology applications.

2.4 Formulation optimization and characterization of drug-loaded nanoparticles

To achieve enhanced drug loading capacity, the study determined a molar ratio of β -lap to PRO as 1:5 for encapsulation within the PLGA core, with an optimal drug-loading ratio of 1:20 (w/w) (Table S1 in the ESM), and the obtained nanoparticle was denoted as $\text{NP}_{\beta\text{-lap+PRO}}$. The surface modification via Gd-TA coordination endowed $\text{NP}_{\beta\text{-lap+PRO}}$ with a marginally expanded hydrodynamic

radius and significantly improved zeta potential stabilization compared to PLGA $_{\beta\text{-lap+PRO}}$ (Fig. 4(a)). Transmission electron microscopy (TEM) further confirmed the nanoparticles' well-defined spherical and monodisperses, possessing a diameter of roughly 107 ± 4.2 nm (Fig. 4(b)). The size of NP $_{\beta\text{-lap+PRO}}$ fell within the ideal range of 50–200 nm, making it suitable for tumor homing and accumulation. In addition, the nanoparticle demonstrated a robust colloidal stability of 72 h across diverse solution systems (Fig. 4(c)), and exhibited good size uniformity (Fig. S7 in the ESM). Importantly, a pH-responsive release profile for both $\beta\text{-lap}$ and PRO was demonstrated by the NP $_{\beta\text{-lap+PRO}}$ system, which was attributed to the gradual decomposition of its rigid shell, especially under acidic conditions. A quantitative evaluation of the release behavior of $\beta\text{-lap}$ and PRO was conducted at endosomal pH (5.5, acetate buffer) and physiological pH (7.4, phosphate buffer) (Fig. 4(d) and Fig. S8 in the ESM). At pH 7.4, the cumulative drug release rates of $28\% \pm 2.7\%$ for $\beta\text{-lap}$ and $27.1\% \pm 3.2\%$ for PRO were observed after 48 h. By contrast, a significantly higher release rate was noted at pH 5.5, with respective release rates of $71.67\% \pm 2.5\%$ and $79.6\% \pm 3.9\%$ for the two drugs within the

first 48 h (Fig. 4(d) and Fig. S8 in the ESM). This pH dependent release characteristic achieved sustained and controllable release characteristics of the system. Collectively, these findings demonstrate that superior colloidal stability and acid-triggered release kinetics position NP $_{\beta\text{-lap+PRO}}$ as a promising drug delivery candidate. Its pH-responsive release mechanism specifically targets the tumor microenvironment, addressing a critical need for precision combination therapies in solid malignancies.

2.5 NP $_{\beta\text{-lap+PRO}}$ potentiated apoptosis of tumor cells through ROS supply and PARP1 degradation

To evaluate the NPs-induced cancer cell death, we first conducted MTT cytotoxicity assays on A549 lung cancer cells. NP $_{\beta\text{-lap+PRO}}$ exhibited a dose-dependent cytotoxicity against A549 cells (Fig. 4(e)). Although non-neoplastic MLE-12 lung epithelial cells displayed dose-responsive cytotoxicity (Fig. S9 in the ESM), A549 adenocarcinoma cells exhibited substantially amplified growth suppression. This differential vulnerability, attributable to cancer-specific NQO1 and PARP1 overexpression, confirms the tumor-selective targeting efficacy. The flow cytometric apoptosis analysis

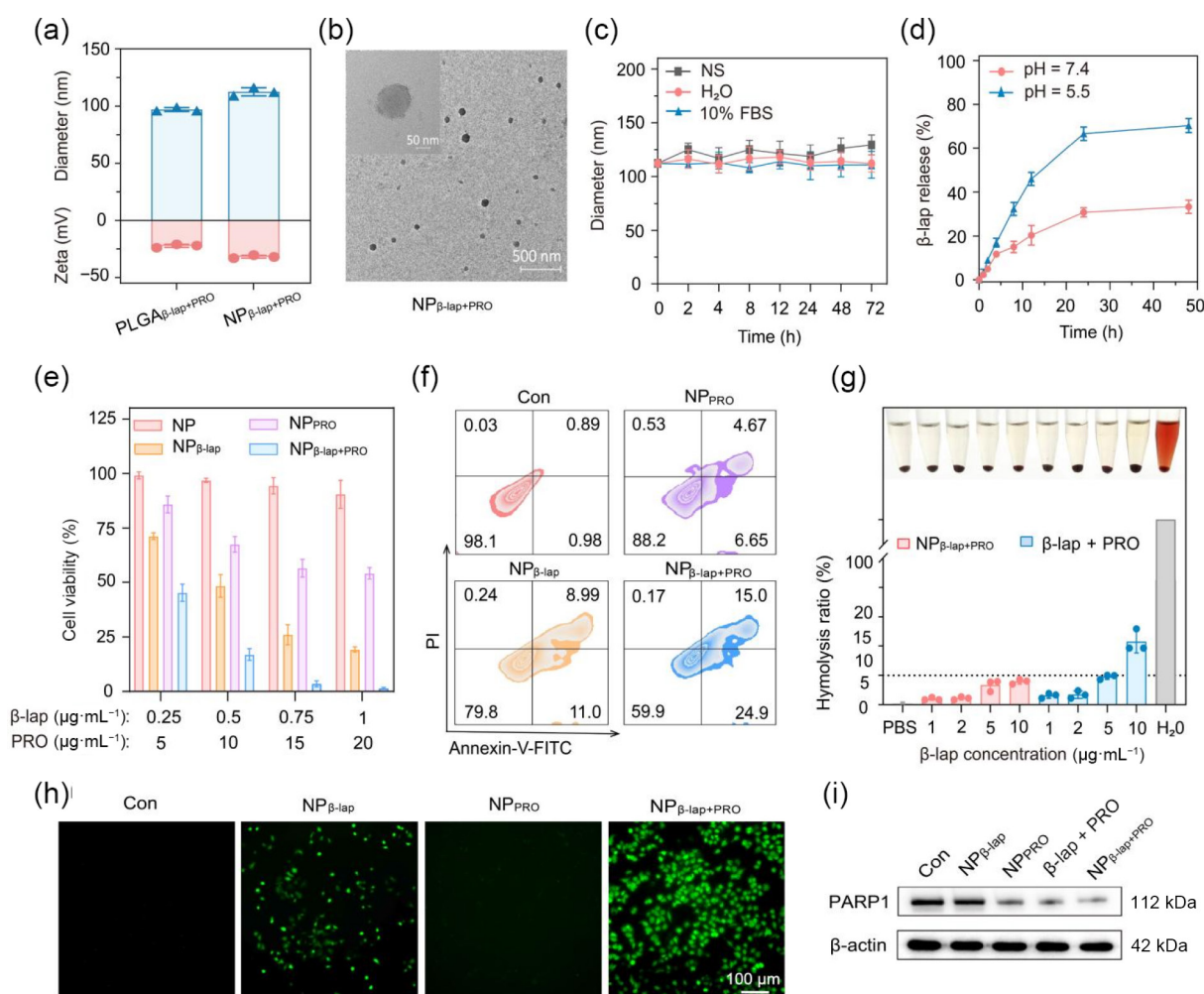


Figure 4 Nanoparticle characterization and *in vitro* evaluation. (a) The size distribution and zeta potential of PLGA $_{\beta\text{-lap+PRO}}$ and NP $_{\beta\text{-lap+PRO}}$. (b) TEM image of NP $_{\beta\text{-lap+PRO}}$. (c) Colloidal stability of NP $_{\beta\text{-lap+PRO}}$ in NS, water, and 10% FBS. (d) Cumulative $\beta\text{-lap}$ release efficiency across neutral (pH 7.4) and acidic (pH 5.5) ranges. (e) The survival capacity of A549 cells under different nanoparticles for 48 h. (f) A549 cells were subjected to different treatments and the apoptosis rate was measured using the membrane associated protein Annexin V-FITC/PI apoptosis assay. (g) Photographs and quantitative results of NP $_{\beta\text{-lap+PRO}}$ and $\beta\text{-lap}$ + PRO membrane-breaking activity on mouse RBCs at varying concentrations. (h) Intracellular ROS generation induced by nanoparticles in A549 cells, as detected by DCFH-DA staining (green). (i) Western blot analysis of PARP1 expression in treated A549 cells. The data are represented as mean $\pm$ SD ($n = 3$).

revealed enhanced apoptotic activity in NP $_{\beta\text{-lap+PRO}}$ -treated cells compared to single-drug formulations after 6 h of incubation (Fig. 4(f) and Fig. S10 in the ESM), confirming synergistic antitumor effects through dual-drug co-delivery. Furthermore, hemocompatibility evaluations under physiological simulation conditions (pH 7.4, 37 °C) showed hemolysis rates below 5% for NP $_{\beta\text{-lap+PRO}}$, contrasting with significant hemolysis induced by free drugs (Fig. 4(g)). This biosafety profile likely stems from hydrophilic modifications on the nanoparticle surface.

The intracellular ROS quantification using DCFH-DA probes revealed substantially elevated ROS levels in NP $_{\beta\text{-lap+PRO}}$ treated cells compared to NP $_{\beta\text{-lap}}$ alone, confirming efficient cellular uptake of the nanoparticles and subsequent induction of oxidative stress cascades (Fig. 4(h) and Fig. S11 in the ESM). Then, the Western blot analysis further assessed the expression and quantification of PARP1 protein (Fig. 4(i) and Fig. S12 in the ESM). After 48 h of treatment of A549 cells with drug-loaded nanoparticles, NP $_{\text{PRO}}$ achieved a PARP1 degradation efficiency of $70.2\% \pm 1.7\%$, whereas NP $_{\beta\text{-lap+PRO}}$ demonstrated the most significant degradation ($75.5\% \pm 2.3\%$). The slight reduction in PARP1 expression observed with NP $_{\beta\text{-lap}}$ treatment was associated with the PARP1 hyperactivation and then depletion, confirming that the PRO-mediated PARP1 degradation plays a key role in enhancing the cell death induced by $\beta\text{-lap}$. Collectively, these findings establish that the nanoparticles facilitate the synergistic drug action by elevating ROS, targeting the PARP1 degradation and apoptotic pathways. Moreover, the pH-dependent release, enhanced anti-cancer potential, and safety evaluation collectively validate the translational potential of this nano-delivery system in lung cancer.

2.6 NP $_{\beta\text{-lap+PRO}}$ for *in vivo* MRI monitoring of subcutaneous tumors

Afterward, we further evaluated the MRI imaging capabilities of NP $_{\beta\text{-lap+PRO}}$ and investigated its tumor accumulation. NP $_{\beta\text{-lap+PRO}}$ generated the concentration-dependent T_1 -weighted imaging (T1WI) contrast enhancement (Fig. 5(a)), evidenced by pseudocolor map transitions from dark blue to red. This validates gadolinium's utility as an MRI tracer for quantitative biodistribution assessment [54]. Next, *in vivo* imaging experiments were performed to observe the distribution of NP $_{\beta\text{-lap+PRO}}$ in A549 subcutaneous tumor model. Following the intravenous administration of NP $_{\beta\text{-lap+PRO}}$ or GdCl₃, A549 tumor-bearing mice were scanned using a Philips Ingenia 3.0T MRI system at 0, 2, 4, 8, 12, and 24 h thereafter (Fig. 5(b)). Subsequently, we quantify the signal-to-noise ratio (SNR) over time in the tumor region and the adjacent muscle tissue following the intravenous injection of GdCl₃ or NP $_{\beta\text{-lap+PRO}}$ (Fig. S13 in the ESM). Although both agents enhanced tumor contrast relative to baseline, NP $_{\beta\text{-lap+PRO}}$ achieved peak signal intensity at 8 h and exhibited sustained retention within the tumor up to 24 h. In contrast, GdCl₃ rapidly cleared from the tumor site, showing the minimal signal by 8 h. These findings highlight the superior tumor accumulation and prolonged the retention of NP $_{\beta\text{-lap+PRO}}$, underscoring its potential for MRI-guided drug delivery and longitudinal tumor imaging.

2.7 *In vivo* antitumor efficacy of NP $_{\beta\text{-lap+PRO}}$ -mediated apoptosis

To assess the antitumor efficacy of the formulations, we established an A549 tumor-bearing mouse model. Mice were randomly

divided into four treatment groups ($n = 5$): G1, NS; G2, NP $_{\beta\text{-lap}}$; G3, NP $_{\text{PRO}}$; and G4, NP $_{\beta\text{-lap+PRO}}$. Each mice received five times administrations from days 0 to 8 (Fig. 5(c)). The tumor growth volume (Fig. 5(d) and Fig. S14 in the ESM), gross morphology photos (Fig. 5(e)), and tumor weight (Fig. 5(f)) collectively demonstrated the therapeutic activity of NP $_{\beta\text{-lap+PRO}}$. Notably, the treatment with NP $_{\beta\text{-lap}}$ and NP $_{\text{PRO}}$ alone inhibited the tumor growth by 62.5% and 37.1%, respectively (Fig. S15 in the ESM). In contrast, the combination therapy NP $_{\beta\text{-lap+PRO}}$ achieved a pronounced tumor growth inhibition rate of 82.4%, underscoring a synergistic antitumor effect (Fig. S15 in the ESM). To further elucidate the underlying mechanism, we analyzed and quantified tumor tissues to assess molecular changes following treatment. The Western blot analysis of tumor tissues (Fig. 5(g) and Fig. S16 in the ESM) revealed significant reduction of PARP1 protein expression in the NP $_{\text{PRO}}$ and NP $_{\beta\text{-lap+PRO}}$ treatment groups, confirming that the observed antitumor activity was associated with the PROTAC-mediated selective degradation of PARP1.

To further validate the intratumoral ROS generation, *in vivo* DCFH-DA fluorescence imaging was conducted. The treatment with NP $_{\beta\text{-lap+PRO}}$ induced a marked increase in intracellular strong green fluorescence signals (Fig. 5(h)), indicating that the PROTAC-mediated PARP1 degradation is the key to ROS generation. Concurrently, elevated levels of $\gamma\text{-H2AX}$ were detected following NP $_{\beta\text{-lap+PRO}}$ administration (Fig. 5(h)), implicating extensive DNA damage with this therapeutic strategy. The combined action of PRO resulted in bypassing the DNA repair mechanism and converted SSBs into lethal DSBs. The TdT-mediated dUTP-biotin nick end labeling (TUNEL) assay staining of tumor tissues further confirmed the superior therapeutic efficacy of NP $_{\beta\text{-lap+PRO}}$ treatment, as evidenced by the highest proportion of green fluorescence TUNEL-positive cells observed in this group compared to controls (Fig. 5(h)). These findings align with current research emphasizing the potential of PROTAC-based approaches in enhancing oxidative stress and compromising genomic stability within tumors. Notably, all treatment groups exhibited steady increases in body weight during the dosing period (Fig. S17 in the ESM), indicative of minimal systemic toxicity. In addition, the hematoxylin-eosin (H&E) staining of major organs (heart, liver, spleen, lung, and kidney) in tumor-bearing mice revealed no detectable histopathological abnormalities across experimental groups, confirming the favorable biocompatibility of the nanoparticle, as detailed in Fig. 5(i). Nonetheless, the limitations of this include the lack of large-scale production capability and the potential for long-term cumulative effects, warranting further investigation and optimization in future studies.

3 Conclusions

In summary, we developed a pH-responsive magnetic nanoparticle platform for the co-delivery of synergistic NQO1-activating therapeutics and PROTACs, addressing key limitations in drug delivery while enhancing intratumoral accumulation. Leveraging its favorable biocompatibility and intrinsic paramagnetic properties, this nanoparticle also enables real-time MRI-guided therapeutic monitoring. The pH-responsive nanoparticle dissociates in the acidic microenvironment of the tumor. The formation of ROS induced by $\beta\text{-lap}$ is NQO1-dependent, whereas the PROTAC-mediated PARP1 degradation preserves NAD(P)H pools to amplify ROS generation. This amplification cycle generates an oxidative

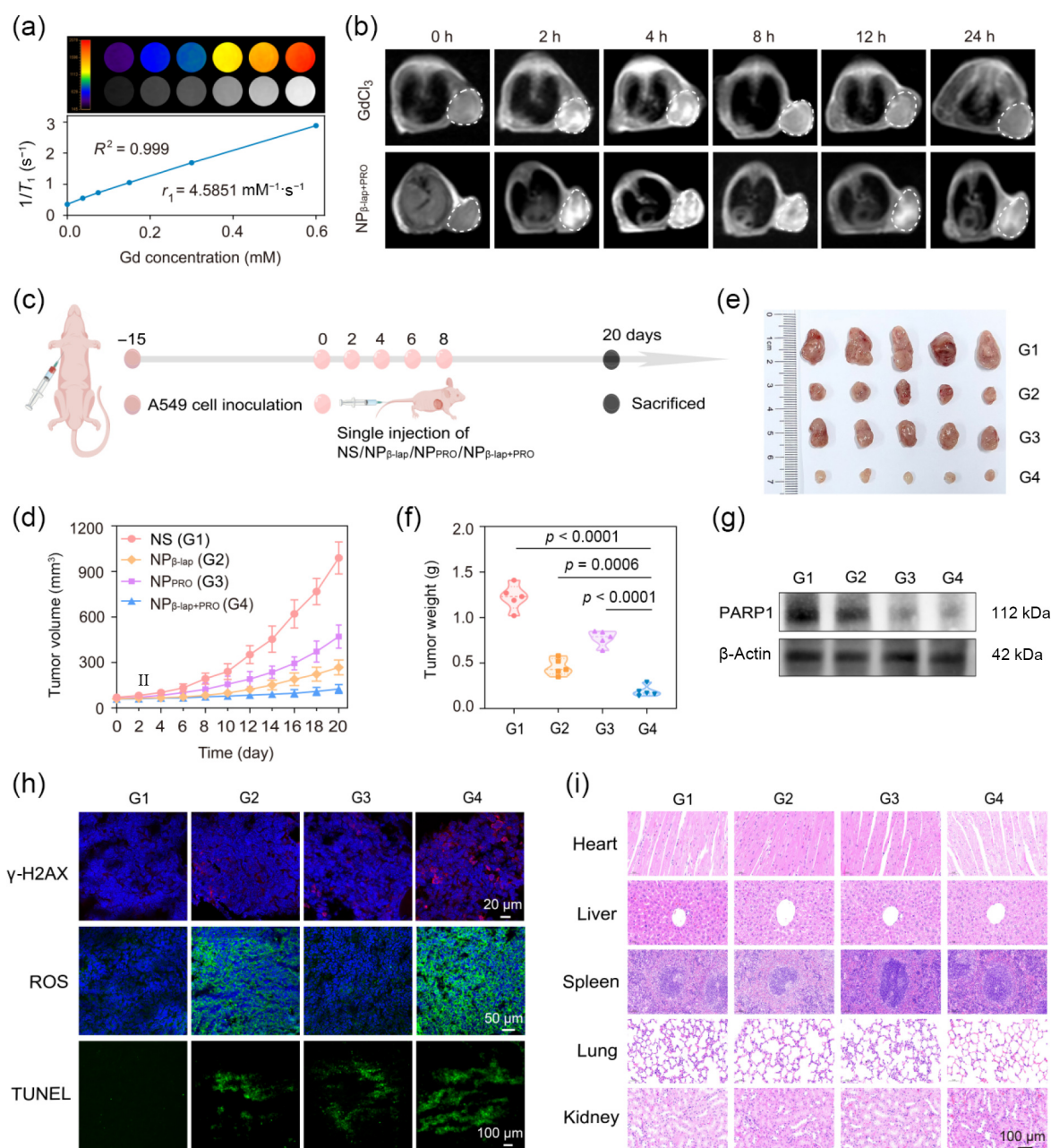


Figure 5 Antitumor efficacy of nanoparticle in A549 tumor-bearing mice. (a) T_1 -weighted pseudocolor MR images at different Gd concentrations and linear fitting of $1/T_1$ versus Gd concentration of $\text{NP}_{\beta\text{-lap+PRO}}$. (b) MRI T_1 -weighted images of the mice that treated with GdCl_3 and $\text{NP}_{\beta\text{-lap+PRO}}$. (c) Schematic illustrating the *in vivo* therapeutic approach in the A549 tumor-bearing mice. (d) The growth curve of the relative tumor volume of each group of mice detected at the specified time. (e) Gross morphology photos of tumor tissue in each group. (f) Weights of tumor tissue in each group. (g) Western blot analysis of PARP1 expression in tumor tissues. (h) Evaluation of immunofluorescence staining of γ -H2AX in tumor tissue, representative ROS generation in tumors using a ROS probe via CLSM, and representative TUNEL staining of tumor sections. (i) H&E staining images of the heart, liver, spleen, lung, and kidney from mice from each group. The data are represented as mean $\pm$ SD ($n = 5$).

burst that induces DNA damage. By exploiting the PROTAC-mediated PARP1 degradation to suppress DNA repair, single-strand breaks are consequently converted into lethal double-strand breaks. This multimodal mechanism culminates in the selective induction of apoptosis and suppression of NQO1-overexpressing NSCLC. Collectively, this study provides a strategy for tumor-targeted therapy through the development of multifunctional nanoparticle delivery systems, while demonstrating the transformative potential of precision oncology in NSCLC treatment.

4 Experimental

4.1 Materials

Gadolinium trichloride hexahydrate ($\text{GdCl}_3 \cdot 6\text{H}_2\text{O}$), TA, olaparib, and PLGA were purchased from Aladdin (China). VHL was purchased from MedChemExpress (Shanghai, China). N,N,N',N'-Tetramethylethylenediamine (TEMED) and DCFH-DA were acquired from Sigma. Dulbecco's modified Eagle's medium (DMEM), trypsin-ethylene diamine tetraacetic acid (EDTA)

(0.25%), and Roswell Park Memorial Institute-1640 (RPMI-1640) were purchased from Gibco BRL (Eggenstein, Germany). FBS was purchased from ExCell Bio (China). DIC and zVAD-fmk were purchased from Macklin. Albumin. Bovine serum albumin (BSA) was obtained from Biofrox (Germany). Cell lysis buffer for Western blotting was purchased from Biosharp (Beijing, China). MTT was purchased from Sigma-Aldrich (Missouri, USA). Polyvinylidene fluoride (PVDF) and Immobilon Western chemiluminescent horseradish peroxidase (HRP) substrate membranes were obtained from Millipore (Shanghai, China). The bicinchoninic acid (BCA) protein assay kit was acquired from Keygen (Guangzhou, China). Anti-PARP1 and anti- β -actin antibody were obtained from Abcam (Beijing, China). All other chemical substances used in the experiment were of analytical grade and could be used without further purification. PAR ELISA kit was purchased from Fine Test (China). The NAD⁺/NADH assay kit with WST-8, the enhanced ATP assay kit, the Caspase-3 activity assay kit, the DNA damage assay kit by γ -H2AX immunofluorescence, the DNA damage comet assay kit, and the colorimetric TUNEL apoptosis assay kit were purchased from Beyotime (Shanghai, China).

4.2 Characterization

NMR spectra were measured using a Bruker ARX 400 NMR spectrometer (Bruker, Billerica, MA, USA) with dimethyl sulfoxide (DMSO- d_6) as the solvent. All solvents used were of high performance liquid chromatography (HPLC) grade. Hydrodynamic characteristics (size distribution and colloidal stability) and surface charge (zeta potential) of the nanoparticles were analyzed with a Malvern Zetasizer Nano ZS90 instrument (Malvern, UK). The UV-3802 spectrophotometer (UNICO, Shanghai, China) was used to record the absorption spectrum. The absorbance and bioluminescence intensity were measured using a multifunctional microplate reader analysis system (Biotek Cytation 5, USA). Western blot images were obtained using a ChemiDoc MP imaging system (Bio-Rad, USA). Flow cytometry analyses were performed on a BD FACS Celesta flow cytometer (BD, USA). Sample microstructural features were examined by transmission electron microscopy (TEM, JEOL JEM-1400 Plus, Japan) at 120 kV accelerating voltage using carbon-coated copper grids. Confocal images were acquired using a confocal laser scanning microscopy (CLSM, ZEISS LSM 880, Germany).

4.3 Cell culture

To grow A549 human lung carcinoma cells, a mixture of RPMI-1640 medium, 10% FBS, 1% penicillin solution, and 1% streptomycin was prepared. Mouse lung epithelial MLE-12 cells were cultured in DMEM medium supplemented with 10% FBS, 1% penicillin, and 1% streptomycin. The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO₂ and 21% O₂.

4.4 In vitro cytotoxicity of drugs and nanoparticles

The cytotoxicity of drugs and nanoparticles on A549 cells was evaluated using MTT assay. A 96-well plate typically contained 3000–5000 cells per well in 0.1 mL of medium. Therefore, the cell suspension concentration was adjusted to 30,000–50,000 cells/mL⁻¹ by adding medium. The plate was placed in an incubator and cultured overnight or for 24 h. The next day, drugs with gradient concentrations were prepared. The old medium in each well was aspirated and discarded, and 0.1 mL of fresh medium was added

per well. Each treatment group was set up with 3–5 replicate wells. Drugs were added to the plate from the lowest to the highest concentration, ensuring that the volume added to each well was as consistent as possible. Then, cells were treated with drugs or nanoparticles of different concentrations. After processing, the culture medium was removed and the cells were washed three times with phosphate-buffered saline (PBS). 10 μ L of MTT solution (5 mg/mL⁻¹) was added to each well and incubated in the dark for 4 h. After incubation, supernatants were aspirated and replaced with 150 μ L of DMSO per well to solubilize formazan crystals, followed by 10 min of orbital shaking to ensure complete dissolution. Finally, the absorbance was measured at 490 nm using a microplate reader.

4.5 Calculation of DC₅₀

A549 cells were seeded in 6-well plates at a density of 5×10^5 cells per well. After overnight culture, the cells were treated with a gradient concentration of the PROTAC (0, 0.625, 1.25, 2.5, 5, 10, and 20 μ M) for 48 h. Following treatment, proteins were extracted and the remaining levels of the target protein were quantified by Western blot. Data were normalized to the untreated control, which was set to 100%. The concentration and corresponding percentage of the remaining protein were then analyzed in GraphPad Prism using a nonlinear regression model (four-parameter dose-response curve) to calculate the DC₅₀ value and its confidence interval.

4.6 Calculation of the combination index

The β -lap toxicity in A549 cells was assessed at the β -lap:PRO ratios of 1:1, 1:5, and 1:10, and the CI based on cell lethality was calculated using MTT assay. The CompuSyn software was used in this study to evaluate the CI of β -lap and PRO as treatment methods. The CI (Eq. (1)) was calculated based on the MTT method to calculate the IC₅₀ value, which could evaluate the overall treatment outcome. When CI was greater than 1, it indicated antagonistic behavior; when CI was 1, it corresponded to additive behavior; and when CI was less than 1, it indicated cooperative behavior.

$$CI = \frac{IC_{50}(X+Y)}{IC_{50}(X)} + \frac{IC_{50}(X+Y)}{IC_{50}(Y)} \quad (1)$$

where $IC_{50}(X) = IC_{50}$ of (β -lap)/P(β -lap) and $IC_{50}(Y) = IC_{50}$ of (PRO)/P(PRO) are the IC_{50} values obtained from each drug, respectively, and $IC_{50}(X+Y) = IC_{50}$ of (β -lap + PRO)/P(β -lap + PRO) is the IC_{50} values of both drugs in combination.

4.7 Cellular uptake

In 6-well plates, A549 cells were inoculated at a density of 5×10^5 cells per well. After overnight cell growth, the cells were co-incubated with Cy5 labeled nanodevices (Cy5 0.2 μ g/mL⁻¹) for 1, 2, 4, and 6 h. The intracellular fluorescence intensity of the processed cells was measured and analyzed using flow cytometry analysis (Accuri C6 Plus, BD Biosciences, USA).

4.8 NAD⁺/NADH, ATP, and PAR quantification

A549 cells were incubated with β -lap, PRO (10 μ M), and β -lap (2 μ M) + PRO (10 μ M) for 4 h, and pretreated for 2 h with or without 50 μ M DIC in a 6-well plate. The NAD⁺/NADH assay kit with WST-8 was then used to determine NAD⁺/NADH levels in accordance with the manufacturer's instructions. A549 cells were cultured using the aforementioned method, and the levels of ATP and PAR in the cells were measured using the enhanced ATP assay kit and PAR ELISA kit, respectively.

4.9 Caspase-3 quantification

Using a similar culture method, A549 cells were pretreated with or without zVAD-fmk (70 μM) for 2 h before being exposed to PBS, β -lap (2 μM), PRO (10 μM), or the combination for 4 h. Caspase-3 activation was then quantified with the Caspase-3 activity assay kit.

4.10 Alkaline comet assays

A549 cells were subjected to an 8-h treatment in 6-well plates, receiving either PBS, individual agents (β -lap at 2 or 5 μM , or PRO at 10 μM), or the combined therapy (β -lap 2 μM + PRO 10 μM). DNA strand breaks in individual A549 cells were quantitatively evaluated via alkaline comet assay (the DNA damage comet assay kit). Specimens were visualized with a Nikon Eclipse TE2000-E fluorescence microscope, and comet tail parameters (DNA in tail (%)) were quantitatively analyzed using Image J.

4.11 The expression of γ -H2AX was detected by immunofluorescence

A549 cells were seeded into a glass-bottom plates at a density of 5×10^5 cells per well and pretreated with or without DIC for 2 h, then exposed to culture medium, β -lap (2 μM), PRO (10 μM), and β -lap (2 μM) + PRO (10 μM) for 8 h. A γ -H2AX immunofluorescence DNA damage detection kit was used to stain the cells for γ -H2AX. Finally, fluorescence was analyzed by CLSM.

4.12 Generation of intracellular ROS

Briefly, A549 cells were plated in 6-well plates at 5×10^5 cells per well and subjected to various treatments, including culture medium (control), β -lap (2 μM), PRO (10 μM), and their combination for 8 h. After treatment, the cells were washed thrice with PBS and incubated with DCFH-DA at 37 $^{\circ}\text{C}$ for 30 min. Fluorescence signals were then visualized and captured under a fluorescence microscope.

4.13 Colony experiment

In a 6-well plate, A549 cells with a density of 100–200 were seeded in one well. A549 cells were incubated with β -lap (2 μM), β -lap (5 μM), PRO (10 μM), and β -lap (2 μM) + PRO (10 μM), and pretreated with or without 50 μM DIC in 6-well plate. After the drug treatment for 48 h, the cells were further incubated for 2–3 weeks. When visible clones appeared in the culture dish, the culture was stopped and the medium was discarded. The cells were washed gently 2–3 times with PBS solution pre-cooled to 4 $^{\circ}\text{C}$, then the PBS was discarded. First, the formed colonies were fixed first with 4% paraformaldehyde for about 20 min, and then they were stained with 0.1% crystal violet solution (Solarbio, G1063, Beijing, China) for 20 min. The colonies were finally photographed and counted.

4.14 Preparation of PLGA@Gd-TA nanoparticles

PLGA was dissolved in acetonitrile (CH_3CN , 10 $\text{mg}\cdot\text{mL}^{-1}$). This solution was then added to ultrapure water (10 mL), followed by sonication of the mixture for 5 min (amplitude 10%, cycle of 5 s on and 2 s off). The nanodevices were prepared by rotary evaporation. After rotary evaporation, a TA solution (40 $\text{mg}\cdot\text{mL}^{-1}$, 100 μL) and a $\text{GdCl}_3\cdot 6\text{H}_2\text{O}$ solution (10 $\text{mg}\cdot\text{mL}^{-1}$, 100 μL) were added, and this process was repeated 3 times. Subsequently, the nanoparticles were purified using dialysis. After the purification was complete, the osmotic concentration was performed using high-molecular-weight

PEG 20000. The concentration process was monitored closely, with the dialysis bag being agitated or rolled regularly to ensure uniform concentration, and the internal volume and the clarity of the solution were checked continually. This process was stopped immediately when the target volume was reached, and the concentrated nanoparticle suspension was recovered into a clean collection tube. Finally, to remove any aggregated nanoparticles formed during the process, the solution was centrifuged at 300g for 2 min, and the supernatant was collected as the final nanoparticle suspension. The suspension was stored at 4 $^{\circ}\text{C}$ for use.

4.15 Preparation of NP $_{\beta\text{-lap+PRO}}$ nanoparticles

Briefly, PLGA (CH_3CN , 20 $\text{mg}\cdot\text{mL}^{-1}$, 1 mL), β -lap dissolved in acetonitrile (CH_3CN , 1 $\text{mg}\cdot\text{mL}^{-1}$, 50 μL), and PRO dissolved in chloroform (CHCl_3 , 2 $\text{mg}\cdot\text{mL}^{-1}$, 500 μL) were added to the ultrapure water (10 mL). The pulsed-mode ultrasonication (10% of amplitude, on for 5 s, and off for 2 s) was performed on the mixture for 5 min in an ice bath (0–4 $^{\circ}\text{C}$) to ensure the thermal stability during emulsification. After sonication, CHCl_3 and CH_3CN were removed from the mixture using a rotary evaporator, yielding PLGA- β -lap+PRO nanoparticles. The resulting solution was allowed to stand in a 4 $^{\circ}\text{C}$ refrigerator for a period of time. Next, under vortex stirring, a TA solution (40 $\text{mg}\cdot\text{mL}^{-1}$, 100 μL) and a GdCl_3 solution (10 $\text{mg}\cdot\text{mL}^{-1}$, 100 μL) were added to the mixture. Following dialysis for purification, the nanoparticles were subsequently concentrated using high-molecular-weight PEG 20000 and collected. We removed any process-induced aggregates by a brief, low-speed centrifugation (300g, 2 min), then collected the supernatant to obtain the final nanoparticle suspension, which was stored at 4 $^{\circ}\text{C}$.

4.16 DLS analysis and TEM images

Using a 633 nm He-Ne laser with 90 $^{\circ}$ collecting optics, DLS (Malvern ZS90, Malvern Instruments Ltd., UK) was used to investigate the size distribution of nanoparticles in a hydrodynamic condition. For TEM analysis, the suspended droplets were placed on a copper mesh, air-dried overnight, and analyzed using TEM.

4.17 Encapsulation efficiency (EE) and drug loading efficiency (DLE)

3 mL of the drug-loaded nanoparticles were loaded into a pre-activated dialysis bag and both ends were clamped tightly. Then, each bag was placed into a container with 30 mL of release medium at either pH 5.5 (acetate buffer) or physiological pH 7.4 (phosphate buffer). The containers were put into a shaker at 37 $^{\circ}\text{C}$, and the shaker was set to 100 rpm and continuous shaking was maintained. At designated time points, 1 mL of the dialysate was collected and 1 mL of fresh release medium was added. A suspension of fresh nanoparticles was freeze-dried using an FD-1 freeze-dryer (Labfreez Instruments Co., Ltd., China). The accurately measured NPs were dissolved in acetone and centrifuged at 2000 rpm for 10 min. The drug loading was determined from supernatant absorbance measurements. The EE and DLE were calculated as follows: $\text{EE} (\%) = W_{\text{drug in supernatant}} / W_{\text{drug in total}} \times 100\%$, and $\text{DLE} (\%) = W_{\text{drug in supernatant}} / W_{\text{total}} \times 100\%$, in which $W_{\text{drug in supernatant}}$ represents the amounts of drug successfully encapsulated in nanoparticles, $W_{\text{drug in total}}$ represents drug equivalents for nanoparticle preparation, and W_{total} represents the total amounts of nanoparticles.

4.18 Apoptosis assay

To investigate cell apoptosis induced by culture medium, NP $_{\beta\text{-lap}}$

(10 μM), NP_{PRO} (10 μM), and NP _{β -lap+PRO} (2 μM) + 10 μM) for 6 h, an Annexin V-FITC/propidium iodide (PI) apoptosis assay was performed. Briefly, A549 cells from each group were PBS-rinsed, then stained with an Annexin V-FITC/PI apoptosis assay for apoptosis detection. In the end, the flow cytometry was applied to figure out the proportion of apoptotic cells.

4.19 Western blot analysis

Cells were seeded into 6-well plates, and A549 cells were subjected to different treatments. Upon the completion of PBS washing, a lysis buffer solution was utilized for cell disruption, followed by protein quantification employing a BCA protein assay kit. Following the separation, the samples were moved to a PVDF membrane after being loaded into an 8% sodium dodecyl sulfate polyacrylamide gel (SDS-PAGE). After blocking the membrane with 5% (w/w) BSA in tris-buffered saline with Tween 20 (TBST) for 2 h, the membrane was thoroughly washed with TBST. Immunoblotting was performed by incubating the membrane with anti-PARP1 and anti- β -actin antibodies at 4 °C overnight. Finally, the membrane was incubated with an anti-goat HRP-conjugated secondary antibody for 2 h and visualized using a chemiluminescence HRP substrate.

4.20 Hemolysis test

The blood compatibility evaluation for β -lap + PRO and NP _{β -lap+PRO} involved the hemolysis test. Succinctly, whole blood was drawn from the orbital venous plexus of mice and deposited in tubes containing anticoagulant tube. Upon the centrifugation to remove the supernatant, the red blood cells (RBCs) were washed three times using PBS and subsequently reconstituted in PBS at 2% hematocrit. A 50 μL of RBC suspension was added to 950 μL of β -lap + PRO and NP _{β -lap+PRO} suspensions at different concentrations. Positive control was deionized water, and negative control was PBS (pH 7.4). Upon 4 h of shaking at 37 °C, the photographic documentation was pictured. Finally, the absorbance measurements of the supernatant were taken at 540 nm via microplate reader. The hemolysis rate was calculated using the following equation: Hemolysis (%) = $(A_{\text{Sample}} - A_{\text{Negative}})/(A_{\text{Positive}} - A_{\text{Negative}}) \times 100\%$, where A_{Sample} refers to the absorbance of sample, and A_{Positive} and A_{Negative} represent the absorbance of the positive control and negative control, respectively.

4.21 MR imaging performance and MR scan parameters

In this experiment, a multisource superconducting MR scanner from the Philips Ingenia 3.0T CX full digital MR system was used, equipped with a DS SmallExtr 8ch 3.0T joint coil (30 $\text{mT} \cdot \text{m}^{-1}$, 100 $\text{T} \cdot \text{m}^{-1} \cdot \text{s}^{-1}$). The scanning sequence included T1WI using a fast spin-echo (TSE) sequence. The scanning parameters for the TSE sequence T1WI transverse scan were as follows: repetition time/echo time (TR/TE): 533/20 ms, slice thickness: 2 mm, slice spacing: 1 mm, field of view (FOV): 80 mm $\times$ 80 mm, and matrix: 256 $\times$ 256. First, the nude mice were numbered and anesthetized via intraperitoneal injection. After successful anesthesia, three animals were placed in a dedicated holder to ensure no movement. The A549 subcutaneous tumor model underwent MRI positioning in the prone orientation; their longitudinal axis was maintained coaxial with both the magnet and the main magnetic field vector during image acquisition. Surrounding partitions were used to minimize air interference and maintain temperature stability. T1WI was performed at 0 (prior to nanoparticle injection), 2, 4, 8, 12, and 24 h post-injection to observe the imaging effects.

4.22 In vivo anticancer analysis

Experimental animals consisted of female BALB/c nude mice (4–6 weeks) supplied by Guangdong GemPharmatech Co., Limited (Guangdong, China), and maintained under specific pathogen-free (SPF) conditions (sterile laminar flow chamber, room temperature 21–25 °C, and humidity of 60%–70%) at the Department of Laboratory Animals, South China University of Technology. All animal handling procedures adhered to both institutional and national laboratory animal care and usage guidelines. To establish A549 subcutaneous tumor model, the nude mice received right armpit injections of A549 cells (5×10^6 cells per mouse) suspended in PBS containing 10% Matrigel. Upon reaching 60 mm^3 volume for subcutaneous tumors, the mice were randomly apportioned into 4 experimental group ($n = 5$): control group (NS), NP _{β -lap} group, NP_{PRO} group, and NP _{β -lap+PRO} group. NP _{β -lap} and NP_{PRO} groups received 10 and 20 $\text{mg} \cdot \text{kg}^{-1}$ β -lap/PRO, respectively, with the NP _{β -lap+PRO} combination administered at equivalent PRO dosage (20 $\text{mg} \cdot \text{kg}^{-1}$). The saline group served as the control. The mice were injected via the tail vein 5 times on days 0, 2, 4, 6, and 8, respectively. On the 20th day, the mice were euthanized, and tumors and important organs were collected. Tumors were harvested for photographing and weighing, then sectioned for H&E and TUNEL staining. Major organs, including the pancreas, lungs, heart, spleen, and kidneys, were also sectioned and stained with H&E for histopathological analysis. The weight of each group ($n = 5$) was measured every 2 days. The tumor volume was calculated: $V (\text{mm}^3) = a \times b^2 \times 0.5$, where a and b are the longest and shortest diameters of the tumor, respectively. To examine the nanoparticle-induced ROS production in tumor tissues, the excised tumors were cryo-embedded in optimal cutting temperature (OCT) tissue matrix (Sakura, Tokyo, Japan), and then a Leica cryostat (Leica CM1900, Germany) was used to section the tissues at 7 μm . The tissues got stained with 10 $\mu\text{g} \cdot \text{mL}^{-1}$ DCFH-DA and Hoechst 33342, and were finally observed by means of CLSM.

4.23 Western blot analysis of tumor tissue

A small amount of tumor tissue was weighed and ground, followed by the addition of lysis buffer. The supernatant was removed by centrifuging the lysate in centrifuge tubes, and then protein quantification was performed via a BCA protein assay kit. The lysate got loaded on an 8% SDS-PAGE and then was blotted to a PVDF membrane. After 2 h of standard blocking (5% BSA in TBST), three TBST washes (5 min each) completed the process. The membrane was incubated with anti-PARP1 and anti- β -actin antibodies at 4 °C (10 h) for immunoblotting. Signal detection was accomplished through chemiluminescent means after 2 h of incubation with a horseradish peroxidase-conjugated goat secondary antibody.

4.24 Immunofluorescence examination of γ -H2AX expression in tumor tissue

After finishing the treatment and harvesting the tumors, the tumors were inserted into the OCT tissue-embedding stuff (Sakura, Tokyo, Japan). The tissues were then sectioned at 7 μm using a Leica cryostat (Leica CM1900, Germany). The tissue sections were stained for γ -H2AX using immunofluorescence staining. Finally, the sections were counterstained with Hoechst 33342 and observed using CLSM.

4.25 Statistical analysis

Every experiment was carried out with at least 3 biological repeats,

and the results were shown as mean $\pm$ standard deviation (SD). Comparisons between groups were analyzed through Student's *t*-test or one-way analysis of variance (ANOVA), with a *p*-value less than 0.05 considered statistically significant. "NS" indicates nonsignificant.

Electronic Supplementary Material: Supplementary material (synthesis steps of PRO, characterization of PRO, cumulative release profile of PRO, and some experimental quantitative data) is available in the online version of this article at <https://doi.org/10.26599/NR.2025.94908191>.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript and the Electronic Supplementary Material. Additional data related to this paper may be requested from the corresponding author upon request.

Acknowledgements

This work was supported by the Key Laboratory of Advanced Interdisciplinary Studies, The First Affiliated Hospital of Guangzhou Medical University of China (No. 2023A03J0355), OpenProject of State Key Laboratory of Respiratory Disease of China (No. SKIRD OP-202311), and Guangdong Provincial Zhong Nanshan Medical Foundation of China (No. ZNS-XS-ZZ-202409-007).

Declaration of competing interest

All the contributing authors report no conflict of interests in this work.

Author contribution statement

S. C. Z.: Data curation, validation, methodology, experimental design, and writing manuscript. C. Z.: Data curation, conceptualization, validation, and writing manuscript. W. C. C. and N. L.: Data curation, conceptualization, and methodology. M. L. J.: Data curation, validation, and methodology. I. U.: Project administration, and writing manuscript. Z. M. L.: Methodology. Y. Y. Y. and X. C. L.: Supervision, conceptualization, project administration, resources, and writing – review & editing. All the authors have approved the final manuscript.

Informed consent

Not applicable.

Ethics statement

All animal experiments were conducted following the established guidelines specified in the Guide to the Care and Use of Laboratory Animals, and the protocol was approved by the Animal Care and Use Committee at the South China University of Technology under accreditation number 2023066.

Use of AI statement

None.

References

- [1] Leiter, A.; Veluswamy, R. R.; Wisnivesky, J. P. The global burden of lung cancer: Current status and future trends. *Nat. Rev. Clin. Oncol.* **2023**, *20*, 624–639.
- [2] Thai, A. A.; Solomon, B. J.; Sequist, L. V.; Gainor, J. F.; Heist, R. S. Lung cancer. *Lancet* **2021**, *398*, 535–554.
- [3] Michels, J.; Adam, J.; Goubar, A.; Obrist, F.; Damotte, D.; Robin, A.; Alifano, M.; Vitale, I.; Olaussen, K. A.; Girard, P. et al. Negative prognostic value of high levels of intracellular poly(ADP-ribose) in non-small cell lung cancer. *Ann. Oncol.* **2015**, *26*, 2470–2477.
- [4] Li, H.; Liu, Z. Y.; Wu, N. Y. Y.; Chen, Y. C.; Cheng, Q.; Wang, J. PARP inhibitor resistance: The underlying mechanisms and clinical implications. *Mol. Cancer* **2020**, *19*, 107.
- [5] Lim, Z. F.; Ma, P. C. Emerging insights of tumor heterogeneity and drug resistance mechanisms in lung cancer targeted therapy. *J. Hematol. Oncol.* **2019**, *12*, 134.
- [6] Wei, X.; Zhou, F. F.; Zhang, L. PARP1-DNA co-condensation: The driver of broken DNA repair. *Sig. Transduct. Target. Ther.* **2024**, *9*, 135.
- [7] Ray Chaudhuri, A.; Nussenzweig, A. The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 610–621.
- [8] Zou, Y. T.; Zhang, H. Q.; Chen, P. Z.; Tang, J. Y.; Yang, S. W.; Nicot, C.; Guan, Z. Y.; Li, X.; Tang, H. L. Clinical approaches to overcome PARP inhibitor resistance. *Mol. Cancer* **2025**, *24*, 156.
- [9] Huang, D.; Kraus, W. L. The expanding universe of PARP1-mediated molecular and therapeutic mechanisms. *Mol. Cell* **2022**, *82*, 2315–2334.
- [10] Jankovic, R.; Radulovic, S.; Arandjelovic, S.; Filipovic, L. Effect of PARP inhibitors on sensitivity of lung and breast cancer cells to trans-platinum as compared to cis-platinum. *J. Clin. Oncol.* **2013**, *31*, e22115.
- [11] Peng, X. P.; Pan, W. Y.; Jiang, F.; Chen, W. M.; Qi, Z. T.; Peng, W. J.; Chen, J. J. Selective PARP1 inhibitors, PARP1-based dual-target inhibitors, PROTAC PARP1 degraders, and prodrugs of PARP1 inhibitors for cancer therapy. *Pharmacol. Res.* **2022**, *186*, 106529.
- [12] Moore, K.; Colombo, N.; Scambia, G.; Kim, B. G.; Oaknin, A.; Friedlander, M.; Lisyanskaya, A.; Floquet, A.; Leary, A.; Sonke, G. S. et al. Maintenance olaparib in patients with newly diagnosed advanced ovarian cancer. *N. Engl. J. Med.* **2018**, *379*, 2495–2505.
- [13] Litton, J. K.; Rugo, H. S.; Ettl, J.; Hurvitz, S. A.; Gonçalves, A.; Lee, K. H.; Fehrenbacher, L.; Yerushalmi, R.; Mina, L. A.; Martin, M. et al. Talazoparib in patients with advanced breast cancer and a germline BRCA mutation. *N. Engl. J. Med.* **2018**, *379*, 753–763.
- [14] Meyer, M. L.; Fitzgerald, B. G.; Paz-Ares, L.; Cappuzzo, F.; Jänne, P. A.; Peters, S.; Hirsch, F. R. New promises and challenges in the treatment of advanced non-small-cell lung cancer. *Lancet* **2024**, *404*, 803–822.
- [15] Lord, C. J.; Ashworth, A. PARP inhibitors: Synthetic lethality in the clinic. *Science* **2017**, *355*, 1152–1158.
- [16] Békés, M.; Langley, D. R.; Crews, C. M. PROTAC targeted protein degraders: The past is prologue. *Nat. Rev. Drug Discovery* **2022**, *21*, 181–200.
- [17] Farley, K.; Bhattacharya, S.; Cleland, J.; Chandran, P.; Wu, J. The targeted protein degradation landscape. *Nat. Rev. Drug Discovery* **2025**, *24*, 164–165.
- [18] Li, Q. R.; Yang, X. Y.; Zhao, M. Q.; Xia, X. L.; Gao, W. H.; Huang, W.; Xia, X. X.; Yan, D. Y. A self-assembled affibody-PROTAC conjugate nanomedicine for targeted cancer therapy. *Nano Res.* **2024**, *17*, 9954–9964.
- [19] Sun, X. Y.; Gao, H. Y.; Yang, Y. Q.; He, M.; Wu, Y.; Song, Y. G.; Tong, Y.; Rao, Y. PROTACs: Great opportunities for academia and industry. *Sig. Transduct. Target. Ther.* **2019**, *4*, 64.
- [20] Chirnomas, D.; Hornberger, K. R.; Crews, C. M. Protein degraders enter the clinic—A new approach to cancer therapy. *Nat. Rev. Clin. Oncol.* **2023**, *20*, 265–278.
- [21] Hu, Z.; Li, R. H.; Cui, X. Y.; Hu, C. J.; Chen, Z. L. Tailoring albumin-based theranostic PROTACs nanoparticles for enhanced NIR-II

- bioimaging and synergistic cancer chemo-phototherapy. *Chem. Eng. J.* **2023**, *469*, 143883.
- [22] Ming, H.; Li, B. W.; Jiang, J. W.; Qin, S. Y.; Nice, E. C.; He, W. F.; Lang, T. Y.; Huang, C. H. Protein degradation: Expanding the toolbox to restrain cancer drug resistance. *J. Hematol. Oncol.* **2023**, *16*, 6.
- [23] Li, X.; Song, Y. C. Proteolysis-targeting chimera (PROTAC) for targeted protein degradation and cancer therapy. *J. Hematol. Oncol.* **2020**, *13*, 50.
- [24] Ding, M. C.; Zong, Q. Y.; Zhang, D.; Ullah, I.; Zhang, X. Z.; Liang, W. H.; Li, X. C.; Bulatov, E.; Yuan, Y. Y. Self-adaptive nanocarriers overcome multiple physiological barriers to boosting chemotherapy of orthotopic pancreatic cancer. *ACS Nano* **2025**, *19*, 662–679.
- [25] Li, L. S.; Bey, E. A.; Dong, Y.; Meng, J. R.; Patra, B.; Yan, J. S.; Xie, X. J.; Brekken, R. A.; Barnett, C. C.; Bornmann, W. G. et al. Modulating endogenous NQO1 levels identifies key regulatory mechanisms of action of β -lapachone for pancreatic cancer therapy. *Clin. Cancer Res.* **2011**, *17*, 275–285.
- [26] Ross, D.; Siegel, D. NAD(P)H:Quinone oxidoreductase 1 (NQO1, DT-diaphorase), functions and pharmacogenetics. *Methods Enzymol.* **2004**, *382*, 115–144.
- [27] Yu, J.; Zhong, B. L.; Zhao, L.; Hou, Y.; Ai, N. N.; Lu, J. J.; Ge, W.; Chen, X. P. Fighting drug-resistant lung cancer by induction of NAD(P)H:quinone oxidoreductase 1 (NQO1)-mediated ferroptosis. *Drug Resist. Updates* **2023**, *70*, 100977.
- [28] Ye, M. Z.; Han, Y. X.; Tang, J. B.; Piao, Y.; Liu, X. R.; Zhou, Z. X.; Gao, J. Q.; Rao, J. H.; Shen, Y. Q. A tumor-specific cascade amplification drug release nanoparticle for overcoming multidrug resistance in cancers. *Adv. Mater.* **2017**, *29*, 1702342.
- [29] Huang, X. M.; Motea, E. A.; Moore, Z. R.; Yao, J.; Dong, Y.; Chakrabarti, G.; Kilgore, J. A.; Silvers, M. A.; Patidar, P. L.; Cholka, A. et al. Leveraging an NQO1 bioactivatable drug for tumor-selective use of poly(ADP-ribose) polymerase inhibitors. *Cancer Cell* **2016**, *30*, 940–952.
- [30] Beg, M. S.; Boothman, D.; Khosama, L.; Arriaga, Y.; Verma, U.; Sanjeeviaiah, A.; Kazmi, S.; Fattah, F.; Pilarski, S.; Rodriguez, M. et al. 708P—A phase I/Ib, multi-center trial of ARQ-761 (Beta-Lapachone) with gemcitabine/nab-paclitaxel in patients with advanced pancreatic cancer. *Ann. Oncol.* **2019**, *30*, v271.
- [31] Gerber, D. E.; Beg, M. S.; Fattah, F.; Frankel, A. E.; Fatunde, O.; Arriaga, Y.; Dowell, J. E.; Bisen, A.; Leff, R. D.; Meek, C. C. et al. Phase I study of ARQ 761, a β -lapachone analogue that promotes NQO1-mediated programmed cancer cell necrosis. *Br. J. Cancer* **2018**, *119*, 928–936.
- [32] Song, S. Y.; Park, H. J.; Yoon, S. M.; Shin, S. S.; Ahn, S. D.; Kim, J. H.; Hong, Y. C.; Choi, E. K. Polymorphism in NQO1 pro187ser and survival of non-small cell lung cancer. *J. Clin. Oncol.* **2005**, *23*, 9596.
- [33] Du, Y.; Yamaguchi, H.; Wei, Y. K.; Hsu, J. L.; Wang, H. L.; Hsu, Y. H.; Lin, W. C.; Yu, W. H.; Leonard, P. G.; Lee, G. R. et al. Blocking c-Met-mediated PARP1 phosphorylation enhances anti-tumor effects of PARP inhibitors. *Nat. Med.* **2016**, *22*, 194–201.
- [34] Dai, X.; Zhu, Y. Q.; Su, M.; Chen, J. B.; Shen, S.; Xu, C. F.; Yang, X. Z. Rigid shell decorated nanodevice with Fe/H₂O₂ supply and glutathione depletion capabilities for potentiated ferroptosis and synergized immunotherapy. *Adv. Funct. Mater.* **2023**, *33*, 2215022.
- [35] Mitchell, M. J.; Billingsley, M. M.; Haley, R. M.; Wechsler, M. E.; Peppas, N. A.; Langer, R. Engineering precision nanoparticles for drug delivery. *Nat. Rev. Drug Discovery* **2021**, *20*, 101–124.
- [36] Xu, Q.; Hu, X. Y.; Ullah, I.; Lin, T. A.; Liu, Y.; Dong, H.; Zong, Q. Y.; Yuan, Y. Y.; Wang, J. Biomimetic hybrid PROTAC nanovesicles block multiple DNA repair pathways to overcome temozolomide resistance against orthotopic glioblastoma. *Adv. Mater.* **2025**, *37*, 2504253.
- [37] Cully, M. Nanoparticles improve profile of molecularly targeted cancer drug. *Nat. Rev. Drug Discovery* **2016**, *15*, 231.
- [38] Becker, M. L.; Burdick, J. A. Introduction: Polymeric biomaterials. *Chem. Rev.* **2021**, *121*, 10789–10791.
- [39] Beach, M. A.; Nayanathara, U.; Gao, Y. T.; Zhang, C. H.; Xiong, Y. J.; Wang, Y. F.; Such, G. K. Polymeric nanoparticles for drug delivery. *Chem. Rev.* **2024**, *124*, 5505–5616.
- [40] Ju, Y. M.; Wang, Z. Y.; Ali, Z.; Zhang, H. C.; Wang, Y. Z.; Xu, N.; Yin, H.; Sheng, F. G.; Hou, Y. L. A pH-responsive biomimetic drug delivery nanosystem for targeted chemo-photothermal therapy of tumors. *Nano Res.* **2022**, *15*, 4274–4284.
- [41] Zheng, X. L.; Song, X. H.; Zhu, G. N.; Pan, D. Y.; Li, H. N.; Hu, J. K.; Xiao, K.; Gong, Q. Y.; Gu, Z. W.; Luo, K. et al. Nanomedicine combats drug resistance in lung cancer. *Adv. Mater.* **2024**, *36*, 2308977.
- [42] Mitragotri, S.; Lahann, J. Materials for drug delivery: Innovative solutions to address complex biological hurdles. *Adv. Mater.* **2012**, *24*, 3717–3723.
- [43] Martins, C.; Sousa, F.; Araújo, F.; Sarmiento, B. Functionalizing PLGA and PLGA derivatives for drug delivery and tissue regeneration applications. *Adv. Healthcare Mater.* **2018**, *7*, 1701035.
- [44] Zuo, X. L.; Chen, Z. Q.; Gao, W.; Zhang, Y.; Wang, J. G.; Wang, J. F.; Cao, M.; Cai, J.; Wu, J. D.; Wang, X. H. M6A-mediated upregulation of LINC00958 increases lipogenesis and acts as a nanotherapeutic target in hepatocellular carcinoma. *J. Hematol. Oncol.* **2020**, *13*, 5.
- [45] Rouleau, M.; Patel, A.; Hendzel, M. J.; Kaufmann, S. H.; Poirier, G. PARP inhibition: PARP1 and beyond. *Nat. Rev. Cancer* **2010**, *10*, 293–301.
- [46] Hage, C. C.; Gebrael, G.; Sayegh, N.; Ozay, Z. I.; Narang, A.; Crispino, T.; Golan, T.; Litton, J. K.; Swami, U.; Moore, K. N. et al. A pan-tumor review of the role of poly(adenosine diphosphate ribose) polymerase inhibitors. *CA Cancer J. Clin.* **2025**, *75*, 141–167.
- [47] Chappidi, N.; Quail, T.; Doll, S.; Vogel, L. T.; Aleksandrov, R.; Felekyan, S.; Kühnemuth, R.; Stoyanov, S.; Seidel, C. A. M.; Brugués, J. et al. PARP1-DNA co-condensation drives DNA repair site assembly to prevent disjunction of broken DNA ends. *Cell* **2024**, *187*, 945–961.e18.
- [48] Sharma, D.; De Falco, L.; Padavattan, S.; Rao, C.; Geifman-Shochat, S.; Liu, C. F.; Davey, C. A. PARP1 exhibits enhanced association and catalytic efficiency with γ H2A. X-nucleosome. *Nat. Commun.* **2019**, *10*, 5751.
- [49] Parvez, S.; Long, M. J. C.; Poganik, J. R.; Aye, Y. Redox signaling by reactive electrophiles and oxidants. *Chem. Rev.* **2018**, *118*, 8798–8888.
- [50] Anderson, J. M.; Shive, M. S. Biodegradation and biocompatibility of PLA and PLGA microspheres. *Adv. Drug Delivery Rev.* **1997**, *28*, 5–24.
- [51] Hui, Y.; Yi, X.; Hou, F.; Wibowo, D.; Zhang, F.; Zhao, D. Y.; Gao, H. J.; Zhao, C. X. Role of nanoparticle mechanical properties in cancer drug delivery. *ACS Nano* **2019**, *13*, 7410–7424.
- [52] Chargari, C.; Maury, P.; Texier, M.; Genestie, C.; Morice, P.; Bockel, S.; Gouy, S.; Ba, M.; Achkar, S.; Lux, F. et al. Theragnostic gadolinium-based nanoparticles safely augment X-ray radiation effects in patients with cervical cancer. *ACS Nano* **2024**, *18*, 16516–16529.
- [53] Cheng, R.; Meng, F. H.; Deng, C.; Klok, H. A.; Zhong, Z. Y. Dual and multi-stimuli responsive polymeric nanoparticles for programmed site-specific drug delivery. *Biomaterials* **2013**, *34*, 3647–3657.
- [54] Kim, H.; Jin, S.; Choi, H.; Kang, M.; Park, S. G.; Jun, H.; Cho, H.; Kang, S. Target-switchable Gd(III)-DOTA/protein cage nanoparticle conjugates with multiple targeting affibody molecules as target selective T₁ contrast agents for high-field MRI. *J. Controlled Release* **2021**, *335*, 269–280.

