Cetuximab-Conjugated Perfluorohexane/Gold Nanoparticles for Low Intensity Focused Ultrasound Diagnosis Ablation of Thyroid Cancer Treatment

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Abstract

Chemotherapeutic efficacy plays a significant role in the development of nanotheranostic systems for drug delivery in tumor cells. In this study, we demonstrate the self-assembly of C225 conjugate, Perfluorohexane/Gold Nanoparticles (Au-PFH-NPs), which results in low-intensity focused ultrasound diagnosis ablation of thyroid cancer treatment. Cetuximab-Conjugated Perfluorohexane/Gold Nanoparticles (C-Au-PFH-NPs) showed excellent stability in water, PBS, and 20% rat serum. Transmission electron microscopy images revealed the effective construction of C-Au-PFH-NPs with commonly spherical assemblies. The incubation of C625 thyroid carcinoma with C-Au-PFH-NPs triggered apoptosis, which was confirmed by flow cytometry analysis. The C-Au-PFH-NPs showed remarkable antitumor efficacy in human thyroid carcinoma xenografts. The histopathological results additionally confirm the achieved outcomes. Furthermore, we successfully examined the efficiency of C-Au-PFH-NPs when using the thyroid carcinoma low-intensity focused ultrasound (LIFUS) diagnostic imaging in vivo. These findings are clear for LIFUS agents with high performing images. It is also identified that different therapeutic purposes will have extensive potential for future biomedical purposes.

1. Introduction

Anaplastic thyroid carcinoma (ATC) is one of the most malignant carcinomas. It is comparatively rare, and is characterized by fast proliferation, neck invasion, and remote metastasis [1–4]. ATC’s severe prognosis is due to the rapid progression of tumors before diagnosis. Current treatment is based on different combinations of chemotherapy, and exterior ray radiation has been unsuccessful in enhancing survival, resulting in an average survival rate of 4 to 6 months and less than 20% survival rate in 12 months [5–8]. Therefore, there are convincing arguments for the development of a new theranostic approach for initial finding and efficient ATC treatment [9–11].

Recently, triggerable drug-charged nanocarriers coupled with multiple internal or external stimuli, such as pH, temperature, ultrasound, laser, and microwave radiation, have been extensively explored for personalized treatment to enable controlled release. They have shown an excellent possibility to deliver enhanced anticancer treatment impact, with decreased systemic toxicity [12–14]. Low-intensity concentrated ultrasound (LIFUS) has been exhaustively researched for tumor treatment along with the use of ultrasound imaging analysis as a potential exterior activate, which is noninvasive and displays significant tissue-penetrating capacity. Particularly, it can significantly increase the efficacy of chemotherapy, avoiding harm to nearby cells, and reducing adversarial side effects [15]. However, the discharge of LIFUS-triggered drugs from nanocarriers and further tumor therapy is still unsatisfactory. This is largely attributable to the comparatively lower accumulation efficacy of nanoparticles-charged nanotransporters at the tumor sites. Accordingly, numerous nanotransporters have been extensively examined to enhance the aggregation of a large number of tumors without causing any side effects [15–17].

Several reports have shown that overexpression of the epidermal growth factor receptor (EGFR) is strongly associated with tumor progression, migration, and invasion. EGFR is common in ATC patients [18].
Antibodies or small molecules based on EGFR immunotherapy can significantly increase the therapeutic effect against ATC. A human, murine chimeric EGFR-targeted monoclonal antibody called cetuximab has shown high empathy in the human EGFR extracellular domain. It inhibits the signals of the epidermal growth factor in cells by delaying usual receptor functions [19–21]. The Food and Drug Administration (FDA) has approved preclinical treatments using cetuximab for the treatment of neck and head carcinoma and colorectal carcinoma with EGFR-expressing cancer tumors. This C225 might be a suitable objective for the structure of nanocarriers to improve the outcome of ATC therapy. Remarkably, some researchers have revealed that for a wide spectrum of cancers, the blend of C225 with CPT-11 equivalents such as Perfluorohexane/Gold Nanoparticles (Au-PFH-NPs) has significant synergistic antitumor effects [22–25], thus, enhancing ATC diagnostics. However, owing to the reduced vascular dispersal of C225 and the hydrophobicity of the Au-PFH-NPs, the nanoparticles’ (NPs) penetrability in the growth and their quantity in the tumor area were inherently imperfect, which greatly debilitated their anticancer efficacy. Fortunately, these problems can be diminished by incorporating Au-PFH-NPs and C225 into one nanotransporter to attain a C225 and Au-PFH-NP combination chemotherapy while simultaneously enabling the targeted capability for nanocarriers [26–29].

Furthermore, monitoring medical imaging is essential for early diagnosis and tumor progression. Numerous researchers have proposed that LIFUS has the potential to achieve concurrent ultrasound (US) and medication transfer, meeting the present need of the initial treatment and the ATC therapy [30–32]. Due to variability and huge dimensions of microbubbles to realize the tumor, the theranostic strategy of conservative US agents, such as microbubbles, demonstrate outstanding imaging capability but are not appropriate for drug delivery. To avoid this problem, we intensively studied phase-changing NPs that could be activated via LIFUS. Phase-changing NPs provide important benefits in tumor theranostics for the supply of tumor US and US-triggered drugs [33–35]. This new strategy offers the possibility of developing a treatment for malignancy and addressing the present theranostic needs, significantly contradicting ATC.

The objective of this study was to modify the C225 nanocarrier to targetly prevent ATC that might accrue in cancer cells, in addition to the enhanced permeability and retention (EPR) effect, through the tumor homing belongings of the C225. The Au-PFH-NP payload release and the LIFUS-triggered synergistic chemotherapy with C225 potentially make the best use of therapeutic efficacy, improving USI, and diminishing the side effects of chemotherapy, as shown in Figure 1. Due to its tremendous biodegradability and biocompatibility, we used a perfluorohexane (PHF core as the shell structure of the nanocarrier. We then synthesized phase-changing NPs with PHF liquid (29°C boiling point). Meanwhile, the Au-PFH-NPs were burdened into the NPs while the C225 was conjugated on the surface of gold nanoparticles, affording (C-Au-PFH-NPs) C225-conjugated Au-PFH-NPs-charged phase transformation. To our knowledge, this is the first work of a LIFUS-mediated C225 modified nanosystem that assimilates tumor targeting both US imagery and US activated drug conveyance to tackle ATC.

2. Experimental Section
The detailed experimental procedures are given in the supporting information.

2.1. Cell culture and nude mice

The Cell Bank of the Chinese Academy of Sciences (Shanghai, China) acquired a human anaplastic thyroid carcinoma line (C643). The cells were grown in medium RPMI-1640 containing 10% FBS and 1% penicillin-streptomycin at 37°C in humidified air with 5% CO₂. At the Laboratory Animal Center of Department of Ultrasound, Harbin Medical University Cancer Hospital (Harbin, China), BALB/C Female both mice and nude mice (balancing about 19g, 25 days) were bought then raised. All animals on our studies were collected from the Harbin Medical University Cancer Hospital Laboratory Animal Center and retained in accordance with rules authorized by the Harbin Medical University’s Animal Ethics Committee (Harbin, China). Furthermore, all animal experimental activities were strictly in line with the policy of the Harbin Medical University’s Institutional Animal Care and Use Committee (IACUC), and this study was endorsed by the IACUC.

In order to start an ATC model in nude mice, C643 cells were collected, splashed thrice with the FBS free medium of RPMI-1640, and subcutaneously inoculated into each mouse's left flank (3 × 10⁷ C643 cells in 150 μL FBS free medium of RPMI-1640 each mice). A Vernier caliper was used to measure the length and width of the tumour and the tumour quantity was considered by the calculation: volume = (length as width × 2)/2.

2. In vitro analysis

2.1. In vitro intracellular uptake C-Au-PFH-NPs

In cultivation dishes, seeded the C643 cells for CLSM at a mass of 1×10⁶ cell mL/dish, grown at 37°C in moistened air comprising 5% CO₂. The cells were spilt into four groups after 24 h of culture: C-Au-PFH-NPs were handled respectively with 10 min and 15 min Dil-labeled C-Au-PFH-NPs (1 mg/mL), and after blocking the cells were washed three times with PBS. Then, Dil-labeled C-Au-PFH-NPs (1 mg/mL) incubated the cells. The cells were washed with PBS three times after 2 h incubation with nanoparticles, fixed with 4 percent paraformaldehyde (200 μL) for 15 minutes, and then gestated by DAPI (10 μg/mL, 200 μL) for 20 min. Lastly, CLSM pictured the dishes [36–38].

2.2.3. In vitro cytotoxicity assay

The CCK-8 assay assessed the cell viability. C643 cells were seeded into 96-well plates (1×10³ cells per well, 100 μL). After 24 hours’ incubation to assess the cell viability Au-PFH-NPs and C-Au-PFH-NPs treated at levels of 10, 5, 2.5, 1.25, 0.625 and 0.312 μM for 24 hours. Au-PFH-NPs and C-Au-PFH-NPs cells were incubated for 24 hours. The positive control used as the untreated C643 cells. The in vitro cytotoxicity assay performed and the calculated made by the company manufactures guidelines.

2.2.4. Apoptosis examinations
The cells were seeded \((4\times10^6 \text{ C643 cells per well, } 1.5\text{mL})\) into a 6-well dish and grown at 37°C in a humidified incubator with 5% CO\(_2\) for 24 hours. The IC\(_{50}\) concentration used by Au-PFH-NPs and C-Au-PFH-NPs. The cell apoptosis assay grouping technique was in accordance with the cell viability assay group. After administering IC\(_{50}\) concentration of the formulations of Au-PFH-NPs and C-Au-PFH-NPs was implemented 2 hours later [39–41].

### 2.2.5. Cell cycle arrest examinations

The cells were seeded \((4\times10^6 \text{ C643 cells per well, } 1.5\text{mL})\) into a 6-well dish and grown at 37°C in a humidified incubator with 5% CO\(_2\) for 24 hours. The IC\(_{50}\) concentration used by Au-PFH-NPs and C-Au-PFH-NPs. The cells were gathered and analyzed in the PI-stained cells after 24 hours of culture, and the percentages of the cells in the G0/G1, S phase, and G2/M phases were evaluated [42–44].

### 2.2.6. In vitro fluorescence imaging in xenografts tumour

A continuous dosage of DiR labeled Au-PFH-NPs and C-Au-PFH-NPs(2 mg/mL, 200μL) was given to C643 tumour-bearing mice. With 1% pentobarbital, all mice were totally narcotized and fluorescence pictures were acquired before injection and 3h, 6h and 24 h post-injection. A vivid fluorescence imaging for tiny animals evaluated the fluorescence intensity changes in the tumour areas in vivo. For ex vivo fluorescence imaging, the significant organs and tumour of one mouse were gathered. In addition, Dil-labeled Au-PFH-NPs and C-Au-PFH-NPs (2.5 mg per mL, 150 μL) were injected through the intravenous of C643 tumour-bearing mice were injected six hours after injection. At the predetermined post-injection moment, tumour matters and significant tissues were gathered, segmented, and ice-covered. DAPI dyeing was conducted in the dark for 5 min after fastening with 4% paraformaldehyde. The biodistribution of Dil-labeled Au-PFH-NPs and C-Au-PFH-NPs was monitored by CLSM [45–47].

### 2.2.7. Therapeutic efficacy of in vivo

When the subcutaneous tumour reaches 100 mm\(^3\) in volume, an antitumour assay was conducted on xenografts of mice carrying anaplastic thyroid cancer. The tumour-bearing mice were arbitrarily split into 3 communities (n-5 per unit): control group(Saline) and free Au-PFH-NPs and C-Au-PFH-NPs were administered by the organizations. Two hundred microliters of the blend was injected with the same dose of Au-PFH-NPs and C-Au-PFH-NPs (1mg/kg) through the tail vein in a 1% saline solution were determined six hours after injection with the US agent filling the investigation with the tumour superficial. Afterward inoculations of C643 cells, 5 consecutive treatments were performed each 72 hours starting on day 20 and ending on day 37. Each mouse's tumour dimensions and weight was recovered every three days, and changes in tumour volume were examined from the relative tumour dimensions \(V/V_0\) (\(V_0\): initial volume prior to treatment), and tumour growth curves were drawn at the same time. On day 37 days, all mice were euthanized and dissected and weighed the tumour masses. In addition, studies in histology and immunohistochemistry were conducted. Sections of the tissue were re-stained with histophalogy [48–50].
3. Results And Discussion

3.1. Characterization of C-Au-PFH-NPs

Having these compounds at hand, we examined the transmission electron microscopy (TEM) analysis of Au-PFH-NPs (1 and 1a zoomview) and C-Au-PFH-NPs (Figures 1 and 2). Their ability to recapitulate the self-assembly behavior in aqueous solutions was tested. For this purpose, we dissolved the C-Au-PFH-NPs prodrugs in dimethylsulfoxide (DMSO 10 mg/mL) and then rapidly injected them into deionized (DI) water under ultrasonication. This procedure allowed us to validate that the solution was transparent and slightly bluish. Electron microscopy revealed that the drug molecules self-assembled to form a spherical nanoparticle structure. The dynamic light scattering (DLS) showed a single peak distribution of the nanoparticles. The average hydrodynamic diameter (intensity) of the compound 1 was ~107.1 nm, and compound 2 was ~108.0 nm (Figure 2B). There is, however, a certain adhesion between nanoparticles formed by the self-assembly of simplesmall-molecule drugs [51–53]. Therefore, we have a miscible liquid with many hydrophobic drugs by combining the prodrug with the appropriate amount of C225 molecules. These nano-assemblies were formed and widely used for in vivo drug delivery, to solve the problem of adhesion and to optimize cancer-specific drug delivery. Then, we measured the stability of C-Au-PFH-NPs with various parameters such as water, PBS, and 20% rat serum, which showed a significantly stable size in various parameters (Figure 2C). Although C-Au-PFH-NPs can self-assemble to form nanoparticles, taken together they may not be sufficiently stable. Therefore, C225 nanoparticles loaded with Au-PFH were investigated further to evaluate their anticancer efficacy in vitro.

3.2. In vitro cell experiments

3.2.1. In vitro intracellular uptake

As illustrated in Figure 3, the much tougher fluorescence derived from Dil-labeled C-Au-PFH-NPs was more concentrated in the C-Au-PFH-NPs group around the cytomembrane of C643 cells compared to the non-target and antagonistic groups [54]. Furthermore, larger quantities of red fluorescence were noted after exposure to the C-Au-PFH-NPs group. These findings indicated that due to the elevated tumor-homing characteristics of C225, the C-Au-PFH-NPs could adhere to C643 cells, and considerably encouraged intracellular uptake of the C643 cells. In the resentment group, the C-Au-PFH-NPs lost the capacity to target the C643 cells because of the congestion of surplus free C225, leading to low levels of C-Au-PFH-NPs around the cells. This demonstrated that the C-Au-PFH-NPs’ desired targeting effectiveness was the outcome of the EGFR-mediated directing capacity.

3.2.2. In vitro cytotoxicity assay

The cell counting kit-8 assay assessed the cell viability of different NP formulations at distinct levels, through a dose-dependent model. As illustrated in Figure 4A, the cell viability of nanoparticles in the analyzed dose range was greater than 80% at 10 mg/mL. The comparatively small and insignificant viability proposed that the elevated biocompatibility of phase-changing NPs was appropriate...
for in vivo application. Reasonably, the cell viabilities of Au-PFH-NPs and C-Au-PFH-NPs decreased considerably as levels of C-Au-PFH-NPs increased. Particularly, the cell viability of the cells treated with C-Au-PFH-NPs was low at the same concentration, implying that the mixture of C-Au-PFH-NPs could boost cytotoxicity synergistically. Cell viability of C-Au-PFH-NPs. The remarkably improved cytotoxicity of C-Au-PFH-NPs may be due to the increased cell membrane permeability caused by the cavitation effect and the improved cell viability of C-Au-PFH-NPs at the objective location, significantly increasing the inhibitory impression of C-Au-PFH-NPs on cell development.

### 3.2.3. Cell apoptosis and cell cycle assays

Next, we evaluated cellular apoptosis. In several groups, total apoptosis (TA) improved as follows: control and Au-PFH-NPs and C-Au-PFH-NPs, respectively (Figure 4B). It should be noted that the apoptosis proportion of Au-PFH-NPs was smaller than that of C-Au-PFH-NPs, whereas it was significantly greater than that of C-Au-PFH-NPs. Cell cycle assays were also conducted to evaluate whether the cell cycle was influenced by the anti-proliferation used by Au-PFH-NPs and C-Au-PFH-NPs. Compared to the control group, a higher percentage of the G2/M phase was perceived in all preserved sets (Figure 4C). The arrest cycle in the C-Au-PFH-NPs G2/M phase was higher than that in the control group but higher than that in the C-Au-PFH-NPs, which is consistent with the results of cytotoxicity and the apoptosis assays mentioned above. An enhanced proportion of the cell cycle in the G2/M phase was detected, and the distinct declaration stages were ascribed to the distinct effects among distinct tumor cells. Hence, the C-Au-PFH-NPs showed an important cell cycle arrest in C643 cells during the G2/M phase impact.

Complete deliberation of the outcomes of the in vitro experiment indicated that nanoparticles could aid as exceptional vehicles for Au-PFH-NPs and C-Au-PFH-NPs. Their combination with C225 enabled the targeted enhancement of cell recognition and endocytosis and enhanced the therapeutic effect of C-Au-PFH-NPs. Furthermore, we maximized the cell proliferation inhibitory effects which may be due to the increased cell membrane permeability caused by cavitation and ultrasound-targeted microbubble destruction (UTMD) effects improved the release of C-Au-PFH-NPs at the objective site, significantly increasing the inhibitory effect on proliferation of the tumor cells.

### 3.4. In vivo fluorescence imaging of xenograft tumors

Fluorescence imaging was implemented at the pre-arranged period opinions to assess the targeting effectiveness and biodistribution of Au-PFH-NPs and C-Au-PFH-NPs in vitro. Compared to the non-targeted group's small fluorescence signal at each stage, the targeted group's accumulating fluorescence signal seemed to be present at the tumor site and peaky at 6 hours at values six times greater than that of the control group (3.94±0.98), sometimes 109 vs. (9.03±1.02) 108 (ps/cm²/sr)/(μW/cm), respectively, (Figure 5A). The intensity of the fluorescence removed the tumor. Significant tissues were examined after 24 h ex vivo. The tumor intensity fluorescence in the selected group was still two-times greater than that in the non-targeted group, (2.99±0.38) sometimes 108 vs. (2.09±0.32) 108 (ps/cm²/sr)/(μW/cm), respectively. There was virtually no distinction between the targeted and non-targeted groups in the intensified
fluorescence in the corresponding bodies. Meanwhile, considerably greater red fluorescence signals were noted in the targeted group's tumor cryosections at 6 h under CLSM following the tumor tissue's ultrathin segment, relative to the fewer red signals in the non-target group. It should be noted that the red fluorescence signals in the tumor cryosections of the Au-PFH-NPs group were significantly better compared to those of the C-Au-PFH-NPs group after irradiation. The distribution of the fluorescence signal in the main organs showed no significant alteration in either group, except in the liver and spleen (Figure 5B and C). The non-targeted group's low fluorescence signals at the tumor site may have resulted from the EPR effect, which facilitated inert combination in tumor tissues. Contrastingly, the improved fluorescence signal of the target group was primarily due to the C225-mediated endocytosis mechanism. Additionally, the C-Au-PFH-NPs could overcome the biological barriers of the tumor. The accumulation of C-Au-PFH-NPs at the objective places was endorsed after microbubble oscillation, cavitation, and destruction. During the process of the oscillation and crash of the acoustic microbubble by the US-targeted microbubble removal impact, the cell membrane could be interrupted, and its permeability was enhanced, allowing the greater accumulation of C-Au-PFH-NPs at the objective locations. These conclusions further confirm that C225 has the potential to precisely carry nanocarriers to tumor cells, preventing them from rapidly reentering into the systemic circulation, allowing extravascular diagnosis and efficient antitumor therapy with an agent.

3.3.7. In vitro ultrasound imaging

Based on the targeted accumulation capacity of Au-PFH-NPs and C-Au-PFH-NPs in tumor cells, we explored the potential of phase-changing nanoparticles to aid as US contrast to improve US imaging and treatment scratches [55–57]. Following the administration of various medicines before LIFUS irradiation, even lower or anechoic contrast improved US signals were observed in each group (Figure 6A). LIFUS was performed in all groups 6 h after the administration of various treatments, at the same time periods with in vivo ultrasound imaging. Expressively sturdier spot-like echo signs slowly accrued in both modes at the tumor places in the treated group, while no evident deviations were detected in the saline group, and only negligible signs appeared in the non-target group. This suggested that C225 eased the direction of the tumor tissue accretion. Large quantities of microbubbles were produced when phase-changing NPs were subjected to ADV at the LIFUS-triggered tumor site, resulting in improved US imaging. However, owing to the absence of C225-mediated targeting capacity, the inadequate ADV of the Au-PFH-NPs and C-Au-PFH-NPs could not effectively improve ultrasound imaging. Furthermore, no obvious enrichment was observed without the LIFUS irradiation in the Au-PFH-NPs, and the C-Au-PFH-NPs alone could not improve ultrasound imaging in vitro, shown in Figure 6B-D. These findings highlighted that C-Au-PFH-NPs were appropriate as ultrasound imaging agents and efficient as in vivo nanocarriers because of their relative stability. This is in line with the outcomes of the ultrasonic imaging, additionally verifying the effectiveness of the beleaguered ultrasonic of C-Au-PFH-NPs slower than that of the potential of LIFUS irradiation and local LIFUS radioactivity to boost the precision of phase-changing C-Au-PFH-NPs.

3.3.8. Therapeutic efficacy invivo
The antitumor efficacy in vivo was explored in the subcutaneous C643 models, to assess the therapeutic efficacy of the mixture of Au-PFH-NPs and C-Au-PFH-NPs in vitro. Numerous pictures of separate groups of mice were drawn to demonstrate the impact of the therapy. (Figure 7A-C). The therapeutic effectiveness was evaluated by tracking changes in each group's tumor volume. It was observed that the tumor in the saline groups was debauched, and there was no significant decrease in the tumor dimensions in the C-Au-PFH-NPs group, indicating that its dose was dependable in vivo, and that the well-known epidermal growth factor was a target for tumor cell identification and treatment. However, the C-Au-PFH-NP accumulation at the tumor site depended solely on the existence of vessel fenestration and vascular leakage, and the inadequate drug release at the tumor site restricted the therapeutic effect. These findings showed that the C-Au-PFH-NPs in nude mice could enhance the therapeutic effect of anaplastic subcutaneous thyroid cancer. Additionally, compared with the control (saline) groups, H&E, procaspase 9 (brown), and cleaved-caspase 3 (brown) expression levels were enhanced. The Ki67 staining and TUNEL assay were used to measure the apoptosis of the tumor in vivo (Figure 7D). Furthermore, while Au-PFH-NPs significantly reduced body weight during the course of the therapy, the use of C-Au-PFH-NPs showed no statistically significant impact on the body weight among all mouse groups.

The above findings clearly highlight that the combination of C-Au-PFH-NPs attained a notable excellent therapeutic effect to counter ATC in nude mice, highlighting the importance of these curative treatments in tumor treatment. This diagnostic approach is preferred for ATC, significantly improving the healing capacity without noticeable side effects.

4. Conclusion

The data presented in this study highlight a strategic rationale for the effectiveness and safety of Au-PFA-NPs. As the synthetic Au-PFA-NPs and C-Au-PFA-NPs are fully biocompatible composites with minimal modifications, the safety risks can be minimized by considering their clinical translation. Furthermore, considering the ability of Au-PFA-NPs to overcome the cetuximab (C225)-conjugated C-Au-PFA-NPs, it was expected that this approach could be an optional therapeutic platform to treat patients with drug-resistant cancer. Lastly, we envision that in addition to taxane agents, this C-Au-PFA-NPs-based approach could be a simple yet broadly applicable strategy to improve tolerance and present a better organized cytotoxic nanotherapeutic approach compared to other antitumor agents.

Declarations

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None

Authors’ contributions

Y.M. and L.W. assisted with NP synthesis and characterization; H. L. assisted with molecular and biochemical analysis; W.C. and X.Z. - assisted with data curation, formal analysis, and validation; Y.L.
assisted with supervised the research.

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**Availability of data and materials**

All data and material are included in the article and its additional files.

**Ethics approval and consent to participate**

All animal experiments were approved by the Ethics Committee of the Harbin Medical University Cancer Hospital in accordance with the guidelines on animal care and use (File No: 2018-3).

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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Figures

Figure 1

Design and NP formulation of C225 conjugates Au-PFH-NPs for safe and efficient in-vivo drug delivery.
Figure 2

Analysis of Au-PFH-NPs and C-Au-PFH-NPs (A) TEM image of Au-PFH-NPs (1 and 1a zoom view) and C-Au-PFH-NPs (2 and 2a zoom view). Scale bars, 100 nm. (B) DLS image of Au-PFH-NPs and C-Au-PFH-NPs. (C) Stability of the Au-PFH-NPs and C-Au-PFH-NPs in 50% water, PBS, and 20% rat serum at 37 °C.

Figure 3

Cellular uptake of C-Au-PFH-NPs at 10 min and 15 min intervals.
Figure 4

(A) In vitro cytotoxicity against C643 thyroid carcinoma cells. (B) Flow cytometry analysis of Au-PFH-NPs and C-Au-PFH-NPs. (C) Apoptosis quantification by flow cytometry analysis. (D) Cell cycle arrest of Au-PFH-NPs and C-Au-PFH-NPs. (E) Quantification of cell cycle arrest.

Figure 5

In vivo pharmacokinetics and biodistribution studies of Au-PFH-NPs and C-Au-PFH-NPs (A) In vivo plasma of the drugs following intravenous injection of Au-PFH-NPs and C-Au-PFH-NPs. (B)
Biodistribution studies of Au-PFH-NPs and C-Au-PFH-NPs. (C) The intensity of the DiR slable Au-PFH-NPs and C-Au-PFH-NPs.

**Figure 6**

(A) Ultrapure sound image. (B) The organs used rats. (C) Average intensity values of Au-PFH-NPs and C-Au-PFH-NPs. (D) organs used ultrasound (Control-without ultrasound and Au-PFH-NPs and C-Au-PFH-NPs 10 min ultrasound exposure).
Figure 7

(A) Body weight and (B) tumor volume (mm³). (C) Morphology of the tumors 21 days after treatment. Tumor volume, tumor weight, and average body weight. (D) Representative HE, TUNEL, Ki67, procaspase, cleaved caspase three analysis of the excised tumors from the treated groups after injection of the drugs. (400x image magnification)

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