Evaluate the inhibition of cytochrome P450 1A1 for enhancing breast cancer chemotherapy with a turn-on fluorescent probe

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ABSTRACT

The incidence rate of breast cancer ranks first in women malignant tumors worldwide, accompanied by a high mortality. The early diagnosis and therapy are important way to reduce the mortality rate of breast cancer in clinical practice. There are urgent needs for anti-tumor strategies with clear evidence and reliable efficacy. Because cytochrome P450 1A1 (CYP1A1) is commonly expressed in breast cancer and has the excellent ability to metabolize xenobiotics, researchers are interested in studying the vital function of CYP1A1 in breast cancer. Herein, we report a BCy-CYP fluorescent probe for imaging of CYP1A1 in cells and in vivo. This probe has outstanding selectivity and high sensitivity for CYP1A1 detection over other biomolecules. Our results reveal that the intracellular CYP1A1 level increase in MCF-7 and MDA-MB-231 cells, and inhibition of CYP1A1 by carnosol efficiently induce apoptosis in the two kinds of breast cancer cells. Thus, CYP1A1 plays a crucial role in breast cancer. The synergistic effect of carnosol and chemotherapy drug is better than chemotherapy drug alone in tumor-bearing mice. CYP1A1 level may be a new biomarker for breast cancer early diagnosis.

1. Introduction

Breast cancer is one of the most common malignant tumors in women worldwide, accompanied by the high morbidity and mortality. According to statistics, 630,000 patients died of breast cancer every year, accounting for approximately 14 % of the total number of tumor-related deaths among women worldwide [1]. Currently, the standard clinical treatments for breast cancer include surgery, chemotherapy, radiotherapy, and immunotherapy [2]. Breast cancers classification based on the expression of ER/PR and HER2 are significant as they represent different clinical and biological outcomes [3]. The most aggressive subtype is triple negative breast cancer (TNBC) that exhibit decreased survival, because no receptor (ER-, PR-, HER2-) is present on these breast tumors. TNBC accounts for approximately 15 % of all breast cancer diagnoses and lacks recognized therapeutic targets. To date, cytotoxic chemotherapy is still the only choose of systemic treatment. Therefore, it is essential to establish a safe and effective treatment for TNBC [4]. Moreover, although hormone therapy has been proven

successful in receptor positive breast tumors (ER+, PR+), relapsed patients often develop resistance to hormonal therapy, leaving these patients with no efficacious treatment options [4]. In this research, we select triple-negative and ER + breast cancers as the study subjects, which most need to improve treatment strategies.

Cytochrome P450 1A1 (CYP1A1), one of the three subtypes of the CYP1 family, is an extrahepatic phase-I enzyme which metabolizes a large number of endogenous and xenobiotic substrates [5]. The recent studies have indicated that CYP1A1 is constitutively expressed in breast tumors [6]. Vinotini has reported the expression levels of multiple xenobiotic-metabolizing enzymes, involving CYP1A1, in 60 cases of breast tumors from newly diagnosed people who had not received adjuvant therapy before. Their results revealed that CYP1A1 levels increased in breast tumor tissues compared to adjacent normal breast tissue [7]. Since CYP1A1 is ubiquitously expressed in breast cancer and has the ability to metabolize different xenobiotics, more and more interests have been displayed to exploit CYP1A1 biological activity for the diagnosis and therapy of breast cancer [8,9]. Therefore, better

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understanding the relationship between CYP1A1 and breast cancer will improve existing treatment strategies and facilitate the development of targeted therapy.

In recent years, scientific researchers have put substantial effort into exploring effective methods to detect CYP1A1. Compared with other reported methods [10,11], fluorescence bioimaging technology has the advantages of real-time monitoring, less damage to biological samples and visual detection. Small-molecule fluorescent probes, has been extensively used as a powerful and indispensably tool to monitor the biomolecules and their metabolic processes in complex living systems, owing to its a variety of advantages in non-destructiveness, effectiveness, high sensitivity, good membrane permeability and high selectivity, as well as rapid and in-situ detection [12–17]. Small-molecule fluorescent probes have versatile chemical structural modifications, making it easier to capture a variety of biological targets. This also allows for conceiving the specific fluorescent probe for a certain enzyme [18–23]. Although CYP1A1 plays a significant role in biological systems, there are limited fluorescent probes for rapid and effective detection of CYP1A1 level in vivo, the comparison of some probe of recent reports was shown in Table S1 in supporting information [24–29].

In present study, we designed a new fluorescent probe BCy-CYP to test the level of CYP1A1 in living cells and in vivo. BCy-CYP probe has a specific CYP1A1 substrate, it has high sensitivity toward CYP1A1 in living systems. Herein, we successfully employ BCy-CYP to monitor CYP1A1 level in MCF-7 and MDA-MB-231 cell lines and their corresponding tumor-bearing mice models. Our results illustrated that the level of CYP1A1 was increased in breast cancer cells. Based on this result, BCy-CYP is the effective small-molecule fluorescent probe for monitoring CYP1A1. The date providing a direct and significant evidence on the correlation between the CYP1A1 and breast cancer in living cells. We further validated that inhibition of CYP1A1 by carnosol efficiently induced cell apoptosis. And the synergy of carnosol and cisplatin had more effectively therapeutic than the treatment with cisplatin alone. To inhibit CYP1A1 has the enormous potential in the treatment of breast cancer for synergistic chemotherapy drug administration. Therefore, BCy-CYP probe may be a valuable tool for early clinical diagnosis and therapy of breast cancer.

2. Experimental methods

2.1. Synthetic procedures of BCy-CYP

4-(Bromomethyl) benzaldehyde (0.2 g, 1 mmol) and Compound a (0.365 g, 1 mmol) were dissolved in ethanol. The mixture was stirred under reflux at 80 °C. After 48 h, the mixture was concentrated under vacuum. The crude product was purified by silica gel chromatography with CH₂Cl₂/CH₃OH (10:1, v/v) as eluent. Finally, BCy-CYP (0.242 g, 63 % yield) was obtained (a yellow product). ¹H NMR (500 MHz, CD₃OD-D₄) δ (ppm): 8.56 (s, 1 H), 8.40–8.38 (d, 1 H), 8.24–8.21 (m, 3 H), 8.13–8.11 (m, 1 H), 8.03–8.01 (m, 1 H), 7.80–7.77 (m, 1 H), 7.69–7.66 (m, 2 H), 7.56–7.53 (m, 2 H), 4.94–4.90 (m, 2 H), 4.57 (s, 2 H), 3.61–3.56 (m, 2 H), 2.07 (s, 6 H), 1.67–1.64 (t, 3 H), 1.25–1.22 (m, 3 H). ¹³C NMR (125 MHz, CD₃OD-D₄) δ(ppm): 182.53, 153.23, 144.98, 139.15, 137.80, 133.92, 133.50, 131.48, 130.55, 129.97, 128.35, 127.93, 127.34, 127.16, 122.95, 112.54, 71.55, 65.91, 54.28, 43.21, 25.31, 14.28, 13.47. HRMS (ESI): *m/z* calcd for C₂₇H₃₀NO⁺ [M]⁺ 384.2322, found 384.3217.

2.2. Cell models

MCF-7 (ERα+/PR+/HER2+), MDA-MB-231 (ER-/PR-/HER2-) cells were used to establish cisplatin cell models. The cells were treated with 25 µg/mL cisplatin individually for 24 h. Carnosol cell models were established using MCF-7, MDA-MB-231 cells by treating with 40 mM carnosol for 8 h. Before imaging, the cells were pretreated with BCy-CYP for 30 min at 37 °C.

Scheme 1. Detection Mechanism of BCy-CYP against CYP1A1.

2.3. In vivo imaging in living mice

All the MCF-7 tumor model were administered an intratumorally injected of BCy-CYP (100 µM, 50 µl in 1:99 DMSO/saline v/v). Eventually, all groups of mice were anesthetized by intraperitoneal (i.p.) injections of 4% chloral hydrate (0.25 mL). Mice images were taken by a Perkinelmer IVIS Lumina XRMS Series III In Vivo Imaging System, with an excitation filter of 550 nm and an emission of 600–700 nm. The results were displayed as the mean standard deviation of 5 independent measurements. Besides, we merged the fluorescence image with the bright field image to clearly show the reaction site of the MCF-7 tumor mice. All mice experimental procedures were performed according to institutional regulations and guidelines for the use and care of laboratory animals and were approved by the Institutional Animal Care and Use Committee in Binzhou Medical University, Yantai. Approval Number: No. BZ2014–102R.

2.4. Tissue preparation

Mice were sacrificed by cervical dislocation at a fixed time of the day and their tumor were excised out immediately, washed in chilled physiological saline and blotted dry. Then the tumor tissue of mice model were prepared to frozen sections and stained with 5 µM BCy-CYP to confirm the amount of CYP1A1.

3. Results and discussion

3.1. The design strategies of probes BCy-CYP

The synthetic approaches of BCy-CYP probe was outlined in Scheme S1. The synthetic details of compounds were shown in the Supporting Information. The mechanism for the probes was shown in Scheme 1. All the compound were characterized by ¹H NMR, ¹³C NMR and HRMS. A benzoindocyanine dye (BCy), as the fluorophore unit, was applied for emitting the signal, which with the emission wavelength was located in 630 nm. In this work, we incorporated the specific recognition moiety of the ethoxy group into fluorophore BCy to obtain the probe BCy-CYP for detection of CYP1A1. CYP1A1 has the dealkylation for *O*-alkyl groups [30], therefore the ethoxy group as response unit of CYP1A1 has specific response and high sensitivity [24]. Ethoxy group in BCy-CYP is directly weakens the fluorescence intensity of BCy; therefore, BCy-CYP exhibits weak fluorescence. Then CYP1A1 recognizes the ethoxy in BD-AChE, the ether bond is cleaved, and BCy-CYP is transformed to free BCy with fluorescence enhancement. The examination mechanism was outlined in Scheme 1. We would real-time monitoring CYP1A1 and providing a direct and significant evidence on the correlation between the CYP1A1 and breast cancer by using BCy-CYP probe. Furthermore, inhibition of CYP1A1 by carnosol efficiently induced cell apoptosis in breast cancer. And the synergy of carnosol and cisplatin induced more effectiveness therapeutic than that treated with cisplatin alone.

3.2. Spectroscopic properties

Under imitated physiological conditions (10 mM HEPES buffer, pH 7.4), the absorption and fluorescence spectra of BCy-CYP (10 µM) were

Fig. 1. Spectral properties of BCy-CYP. a) Absorption spectra of BCy-CYP and BCy-CYP + CYP1A1. The black curve was the absorption spectrum of BCy-CYP. The red curve was recorded after treatment with 200 nM CYP1A1. b) Fluorescence emission spectra changes of BCy-CYP (10 μ M) with various concentrations of CYP1A1 (0, 20, 40, 60, 80, 100, 120, 140, 160, 180 and 200 nM). c) The plot of the linear relationship between the relative fluorescence intensity at 625 nm and concentrations of CYP1A1 (0–200 nM). d) Plot of fluorescence intensity of BCy-CYP (10 μ M) against the reaction time in the presence of varied concentrations of CYP1A1 (0 and 200 nM). e) Fluorescence responses of BCy-CYP (10 μ M) to a series of CYP isoforms (200 nM) for 30 min and CYP1A1 (200 nM) after added for another 30 min at 37 $^{\circ}$ C: 1. Blank, 2. 1A2, 3. 1B1, 4. 2A6, 5. 2A13, 6. 2B6, 7. 2C8, 8. 2C9, 9. 2D6, 10. 2E1, 11. 3A4. f) Fluorescence responses of BCy-CYP (10 μ M), to 10 μ M various enzymes: 1. Blank, 2. butyrylcholine esterase, 3. pepsin, 4. horseradish peroxidase, 5. nitroreductase, 6. glutathione S-transferases, 7. alkaline phosphatase, 8. monoamine oxidase A, 9. monoamine oxidase B, 10. β -galactosidase, 11. BSA, 12. Glucose. λ_{ex} = 550 nm, λ_{em} = 625 nm. The measurements were performed in 10 mM HEPES (pH 7.4) at 37 $^{\circ}$ C. (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

detected. The results presented in Fig. 1a, the maximum absorption wavelength of BCy-CYP was at 440 nm. In the presence of 200 nM CYP1A1, the maximum absorption peak shifted to 500 nm. Under the condition of CYP1A1 concentration range of 0–200 nM, BCy-CYP fluorescence titration was determined. As presented in Fig. 1b, the relevance fluorescence emission profiles increased with the peaks centered at 625 nm. The excitation wavelength of probe was 500 nm. The linear concentration-dependent fluorescence response range between BCy-CYP and CYP1A1 was 0–200 nM (Fig. 1c). The equation obtained from the calibration curve was $F_{625\text{ nm}} = 2.7947 [\text{CYP1A1}] \text{ nM} + 35.2724$ ($r = 0.9941$). Under the experimental conditions, the detection limit of BCy-CYP toward CYP1A1 was calculated to be 2.7 nM ($3\sigma/k$), suggesting sensitivity highly sensitive of BCy-CYP probe towards CYP1A1 examination. These results investigated that BCy-CYP probe could be available for qualitatively and quantitatively test of CYP1A1.

3.3. Kinetic investigations of BCy-CYP toward CYP1A1

With the synthetic probe, we then evaluated BCy-CYP toward CYP1A1 under imitated physiological conditions (10 mM HEPES buffer, pH 7.4). As illustrated in Fig. 1d, we assessed the reaction activity of CYP1A1 towards BCy-CYP. BCy-CYP fluorescence kinetic curves with different concentrations of CYP1A1 (0, 200 nM) was studied within 30 min. It indicates that CYP1A1 higher concentration emerged a faster reaction and a larger fluorescence enhancement. The fluorescence signal could reach the plateau within 25 min with the CYP1A1 concentration was at 200 nM. These results suggest that BCy-CYP displayed a very quick response to CYP1A1. The rapid response behavior induces BCy-CYP probe a unique capability for real-time bioimaging of intracellular CYP1A1.

3.4. Selectivity of BCy-CYP towards CYP1A1

We next examined the fluorescence response of BCy-CYP toward a series of CYP isoforms to evaluate the selective of BCy-CYP. As shown in Fig. 1e, a remarkable change in the fluorescence intensity among all the detected CYP isoforms after added CYP1A1. After added CYP1A1, compared to CYP1A2, CYP1B1, CYP2B6, CYP2D6 and other CYPs (include 1B1, 2A6, 2A13, 2B6, 2C8, 2C9, 2D6, 2E1 and 3A4), there was ~ 2.5 -fold, ~ 4 -fold, ~ 5 -fold, ~ 4 -fold and ~ 10 -fold difference in fluorescence intensity for CYP1A1, respectively. Furthermore, as presented in Fig. 1f, BCy-CYP probe was incubated with various types of enzymes (butyrylcholine esterase, pepsin, horseradish peroxidase, nitroreductase, glutathione S-transferases, alkaline phosphatase, monoamine oxidase A, monoamine oxidase B, β -galactosidase), BSA and glucose. The results certainly indicated that only added CYP1A1, a remarkable increase of fluorescence emission could be observed. In conclusion, these results revealed that BCy-CYP was suitable for the sensitive detection of CYP1A1 with excellent selectivity, and the excellent responsiveness of BCy-CYP towards CYP1A1 was not affected common biological various enzymes exist in cells or tissues.

3.5. Imaging of CYP1A1 in living cells

Since BCy-CYP probe exhibited greater selectivity towards CYP1A1, we then explored the application of BCy-CYP probe for test of CYP1A1 in living cells. The breast cancer cells utilized in this study included MCF-7 and MDA-MB-231 cells with MCF-10A (normal breast epithelial cell line) as control. MTT assays was performed to investigate the cytotoxicity of different concentrations (from 0 to 100 μ M) of BCy-CYP probe in MCF-7 cells and MDA-MB-231 cells. The results fully revealed that BCy-CYP probe exhibited low cytotoxicity to MCF-7 and MDA-MB-231 living cells (Figs. S3 and S4).

All the experimental cells were incubated with BCy-CYP at 37 $^{\circ}$ C, the

Fig. 2. Laser scanning confocal microscopy images and flow cytometry analysis to assess the changes of CYP1A1 levels in vitro. a) Real-time CYP1A1 imaging with BCy-CYP (5 μ M) in three kinds cells (MCF-10A, MCF-7 and MDA-MB-231) at different points in time: 0, 10, 20, and 30 min via laser scanning confocal microscope as control (Scale bars: 20 μ m). b) Flow cytometry analysis three kinds cells (MCF-10A, MCF-7 and MDA-MB-231) a). c) Mean fluorescence intensity of MCF-10A cells, MCF-7 cells and MDA-MB-231 cells in a). d) Western Blot analysis CYP1A1 level changes in breast normal cells (MCF-10A cells), breast cancer cells (MCF-7 and MDA-MB-231 cells). GAPDH was utilized as internal control. e) Quantitative of CYP1A1 expression in d). Red channel: λ_{ex} = 550 nm, λ_{em} = 600 - 700 nm. Images represent n = 5 independent experiments. (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

fluorescence changes were quantified by flow cytometry, and imaging by laser scanning confocal microscopy. The fluorescence of three types of cells in Fig. 2a gradually increased in the 30 min experimental process. The fluorescence of two kinds of breast cancer cells was significantly strong compared to MCF-10A cells, investigating the higher CYP1A1 level in human breast cancer cells. The bright-field images of Fig. 2a were presented in Fig. S5. Flow cytometry assay was further carried out to clarify the above experimental results (Fig. 2b). The average fluorescence intensity of three types of cells was displayed in Fig. 2c. Additional, the changes of CYP1A1 level was clarified by western blot assay. The results obtained from western blot analysis were consistent with the fluorescence images results and flow cytometry assay (Fig. 2d and e). These experiments disclosed that CYP1A1 level in breast cancer cells are higher than that in breast normal cells, and the probe BCy-CYP could be utilized as a facilitative and effective tool to directly detect CYP1A1 level.

3.6. Inhibition of CYP1A1 increases apoptosis

To further analysis the relationship between CYP1A1 and breast cancer, we employed a pharmacologic method to decrease CYP1A1 levels. In general, cisplatin and other platinum-based compounds are considered as cytotoxic drugs which kill cancer cells by damaging DNA, inhibiting DNA synthesis and mitosis, and inducing apoptotic cell death. Drug metabolism is known to be catalyzed by a range of drug-metabolizing enzymes including the cytochromes P450 (CYPs) [31]. The CYP1A1 have the ability to catalyze the oxidation of foreign chemicals, including a wide variety of anticancer drugs [32]. Carnosol inhibits the AhR which regulates the basal and inducible expression level of CYP1 family members, including CYP1A1 [33]. Previous studies have been demonstrated that carnosol reduce CYP1A1 level in prostate cancer lines and in premalignant bronchial and tongue lines [34,35]. We next investigated the effects on two different breast cancer cells after using carnosol to lower the CYP1A1 level. Before imaging, all the experimental cells were incubated with BCy-CYP for 30 min at 37 $^{\circ}$ C.

Fig. 3. The changes of CYP1A1 levels in MCF-7 and MDA-MB-231 cells. a) MCF-7 and MDA-MB-231 cells were pretreated with 40 mM carnosol for 8 h, respectively. Then confocal microscopy images of CYP1A1 level with BCy-CYP (5 μM) in cells for 30 min (Scale bars: 20 μm). b) Flow cytometry analysis of a). c) The apoptosis rate of MCF-7 and MDA-MB-231 cells were analysed with or without carnosol. d) Western blot analysis CYP1A1 changes in MCF-7 and MDA-MB-231 cells with or without carnosol. GAPDH was utilized as internal control. e) Quantitative of CYP1A1 expression in d). Statistical analysis was performed using Student's t-test: **p < 0.01. Red channel: λ_{ex} = 550 nm, λ_{em} = 600 - 700 nm. Data are presented as mean $\pm$ SD (n = 5). (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

The Annexin V/PI assay was performed to evaluate the apoptotic rate. MCF-7 and MDA-MB-231 cells were pretreated with 40 mM carnosol for 8 h in order to lower the CYP1A1 level before imaging. The fluorescence intensity in Fig. 3a decreases with the addition of carnosol, illustrating that CYP1A1 level in cells decreased. The results received from flow cytometry analysis in Fig. 3b showed the satisfactory consistency. The mean fluorescence intensity of two types of cells was displayed in Fig. 3d. Furthermore, we carried out western blot analysis to clarify the variation of CYP1A1 level, which further revealed the effective suppression of carnosol to CYP1A1 (Fig. 3e and f). As shown in Fig. 3c, after adding carnosol, the apoptosis rate of breast cancer cells increased, validating that carnosol might had a good therapeutic effect for breast cancer. Our results fully indicated that inhibition of CYP1A1 efficiently induced apoptosis in breast cancer cells. Therefore, the inhibition of CYP1A1 by carnosol was beneficial to the relief of breast cancer.

3.7. A new synergistic therapeutic strategy for breast cancer

Encouraged by the experimental results that inhibition of CYP1A1 efficiently increased apoptosis rate of breast cancer cells, we next devoted efforts to investigate the adjunctive therapeutic effect of inhibition of CYP1A1 by carnosol, and synergistic therapeutic effect of chemotherapy drug with carnosol. Cisplatin and its derivatives are vital drugs in the treatment for a wide range of human cancers [36], including breast cancer, testicular cancer, neck and head cancer, and others [37]. Thus, we chose cisplatin as an effective treatment for breast cancer. As indicated in Fig. 4a, the two different breast cancer cells were respectively divided into four groups. The first groups were selected as the control. The second cell groups were added 25 μg/mL cisplatin individually for 24 h before imaging. The third cell groups were treated with 40 mM carnosol for 8 h before imaging. The cells in the fourth groups were co-treated with 40 mM carnosol for 8 h and 25 μg/mL cisplatin for

24 h as introduced in the above groups. As expected, the results of fluorescence intensity could be displayed in an order: control group > cisplatin group > carnosol group > carnosol + cisplatin group (Fig. 4a). These results revealed the fluctuation of CYP1A1 levels and the CYP1A1 levels in carnosol + cisplatin group drops the most. The level of CYP1A1 is slightly reduced after adding cisplatin, which may be caused by a decrease in the number of living cells. The fluctuation of CYP1A1 levels in the four groups was also verified by western blot (Fig. 4d and 4e). We further employed flow cytometry assay to test the changes of fluorescence intensity (Fig. 4b). Besides, flow cytometry was applied to check the apoptosis rate of cells (Fig. 4c). The apoptosis rate of synergistic carnosol and cisplatin were monitored with significant increase than carnosol or cisplatin alone for two types of breast cancer cells, certifying that the synergistic therapy of carnosol and cisplatin displayed more effective therapeutic than only treated with carnosol or cisplatin alone. These results implied that the inhibition of CYP1A1 might consider being a novel synergistic therapeutic method for breast cancer.

3.8. Imaging of CYP1A1 in tumor-bearing mice

Due to our probe BCy-CYP were displayed to be efficacious in living cells, we next studied its efficiency in tumor-bearing mice. The application of BCy-CYP for imaging in vivo was investigated in MCF-7 tumor-bearing mice. Four groups of tumor-bearing mice were chosen for carrying out the therapeutic test: in the first group, the tumor-bearing mice injected via tail vein with saline as the control. The second group were orally administered a 30 mg/kg carnosol once a day for 4 weeks. In the third group, cisplatin (0.5 mg/mL $\times$ 0.5 mL) was given by intraperitoneal injection every other day for 4 weeks. The fourth group was administered with carnosol and cisplatin as described in the second and third groups. We first measured the CYP1A1 level in vivo and ex vivo with BCy-CYP. After intratumorally injected BCy-CYP for 1 h, the tumor-

Fig. 4. The changes of CYP1A1 levels in MCF-7 and MDA-MB-231 cells. a) Confocal microscopy images of CYP1A1 level with BCy-CYP (5 μM) in MCF-7 and MDA-MB-231 cells for 30 min. The first cell group were untreated. The second group were treated with 25 μg/mL cisplatin individually for 24 h before imaging. The third cell groups were treated with 40 mM carnosol for 8 h before imaging. The fourth groups were co-treated with 40 mM carnosol for 8 h and 25 μg/mL cisplatin for 24 h, respectively (Scale bars: 20 μm). b) Flow cytometry analysis of a). c) Apoptosis analysis of two kinds of breast cancer cells as introduced in a). d) Western blot analysis CYP1A1 changes in MCF-7 and MDA-MB-231 cells. GAPDH was utilized as internal control. e) Quantitative of CYP1A1 expression in d). Statistical analysis was performed using Student's t-test: **p < 0.01. Red channel: λ_{ex} = 550 nm, λ_{em} = 600 - 700 nm. Data are presented as mean $\pm$ SD (n = 5). (For interpretation of the references to colour in the Figure, the reader is referred to the web version of this article).

bearing mice were subjected to in vivo imaging system for CYP1A1 detection. Under different treatment conditions, the fluorescence intensities were gathered in the order: control group > cisplatin group > carnosol group > carnosol + cisplatin group (Fig. 5a). The variation of the CYP1A1 levels in tumors were as expected, which revealed the effective inhibition of carnosol to CYP1A1 in mice. The mean fluorescence intensity of Fig. 5a was displayed in Fig. 5e. Fluorescence imaging of the isolated tumors and tumor slices was performed in vitro, which corresponded to the imaging results of mice in vivo (Fig. 5b and d). We carried out H&E pathological staining to clarify the tumor tissue (Fig. 5c). These above results revealed that the probe BCy-CYP had an outstanding ability to detect CYP1A1 levels in vivo. The probe had a significant potential value for clinical auxiliary diagnosis of breast cancer. Additionally, the tumors sizes and body weights of tumor-bearing mice were measured every two days during the 16 day period. As indicated in Fig. 5f, g and h, compared with carnosol or cisplatin alone, the synergistic therapy of carnosol and cisplatin displayed better inhibitory effects on tumor growth, disclosing that the synergistic therapy of carnosol and cisplatin had greater therapeutic effects. Therefore, in terms of overall therapeutic effect, inhibition of CYP1A1 by carnosol and cisplatin would be given priority as one of the adjuvant treatments for breast cancer.

4. Conclusion

In this study, we synthesized a fluorescent BCy-CYP probe to detect the changes of CYP1A1 level in breast normal and cancer cells, and in tumor-bearing mice. Through the using of BCy-CYP, we verify that the intracellular CYP1A1 plays crucial roles in breast cancer. Our experimental results certify that the CYP1A1 level in breast cancer cells are higher than in normal cells, which provide the basis for using BCy-CYP to detect cancerous tissue and normal tissue. To explore the influence of changes in CYP1A1 level on the treatment of breast cancer, we further confirm that inhibition of CYP1A1 by carnosol efficiently induced apoptosis in breast cancer cells. And we disclose that the synergistic therapy of carnosol and chemotherapy drug cisplatin display more effective therapeutic than treatment with only carnosol or chemotherapy drug. BCy-CYP probe is a new chemical tools to accurately diagnose breast cancer. CYP1A1 may be a useful indicator for early detection of breast cancer.

CRedit authorship contribution statement

Yan Huang: Conceptualization, Validation, Formal analysis, Investigation, Writing - original draft. **Lei Yu:** Data curation, Methodology, Investigation, Software. **Pengpeng Lu:** Conceptualization, Software,

Fig. 5. Effects of synergistic therapy of carnosol and cisplatin in the MCF-7 tumor-bearing mice. a) Imaging test of CYP1A1 levels in tumor-bearing mice. The first group were injected via tail vein with saline as the control. The second group were orally administered a 30 mg/kg carnosol once a day for 4 weeks. The third group, cisplatin (0.5 mg/ml $\times$ 0.5 mL) was given by intraperitoneal injection every other day for 4 weeks. The fourth group was administered with the same content of carnosol and cisplatin as described in the second and third groups. All the mice were intratumorally injected with BCy-CYP (100 μ M, 50 μ L in 1:99 DMSO/saline, v/v) for 30 min. b) Imaging of tumor slices of a). c) H&E staining of MCF-7 tumor in four groups. d) The ex vivo fluorescent imaging of dissected heart, liver, spleen, lung, kidney and tumor tissue in four groups. e) Mean fluorescence intensity of the images a). f) The relative tumor volume of subcutaneous mice in four groups. g) The body weight of mice in different groups. Statistical analysis was performed using Student's t-test: ** $p < 0.01$, * $p < 0.005$. h) The photos of tumor tissue in every groups. Fluorescence imaging channel: $\lambda_{ex} = 550$ nm, $\lambda_{em} = 600 - 700$ nm. Data are presented as mean $\pm$ SD ($n = 5$).

Visualization, Writing - review & editing. **Yinghui Wei:** Validation, Formal analysis, Investigation, Validation. **Xiaoyan Wang:** Conceptualization, Funding acquisition, Methodology, Writing - review & editing. **Lingxin Chen:** Funding acquisition, Conceptualization, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.snb.2021.130233>.

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