Engineering naphthalimide-cyanine integrated near-infrared dye into ROS-responsive nanohybrids for tumor PDT/PTT/chemotherapy

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A B S T R A C T

Photodynamic (PDT) and photothermal therapies (PTT) are emerging treatments for tumour ablation. Organic dyes such as porphyrin, chlorin, phthalocyanine, boron-dipyrromethene and cyanine are the clinically or preclinically used photosensitizer or photothermal agents. Development of structurally diverse near-infrared dyes with long absorption wavelength is of great significance for PDT and PTT. Herein, we report a novel near-infrared dye ML880 with naphthalimide modified cyanine skeleton. The introduction of naphthalimide moiety results in stronger electron delocalization and larger redshift in emission compared with IR820. Furthermore, ML880 is co-loaded with chemotherapeutic drug into ROS-responsive mesoporous organosilica (RMON) to construct nanomedicine NBD@ML@RMON, which exhibits remarkable tumor inhibition effects through PDT/PTT/chemotherapy in vivo.

1. Introduction

Near Infrared (NIR) dyes are widely employed in biomedicine, such as fluorescent probes [1], fluorescence-guided surgery [2], photoacoustic imaging [3], phototherapy [4–6], and other fields. Phototherapies, including photodynamic and photothermal therapy, have the advantages of high temporal and spatial accuracy, simple operation and high specificity. Therefore, they are considered as emerging treatments for various tumour ablations. Porphyrin, chlorin, phthalocyanine, boron-dipyrromethene and cyanine derivatives are frequently applied as phototherapeutic agents in preclinical and clinical trials, such as verteporfin [7], bromoazacypirromethene ADP06 [8], indocyanine green (ICG) [9,10]. Nevertheless, the development of structurally diverse near-infrared dyes is of great significance and faces many challenges. The common strategies to construct new NIR dyes include conjugated chain extension, efficient auochrome utilization, and atom substitution of fluorophore core [11,12]. Yang developed a new class of fluorophores named ECX with a five-fused ring, achieving about 300 nm red-shift absorption and emission compared with normal rhodamine [13]. Qian first reported silaanthracene dye, which substituted the oxygen atom with silicon on the rhodamine framework, revealing a 90 nm red-shift [14]. Besides, the covalent connection of traditional dyes is a simple and efficient method to extend wavelength, while retaining the characteristics of traditional dyes. Anthony Romieu’s group combined...
coumarin and hemicyanine to construct a new type of water-soluble NIR fluorophore [15]. Lin integrated rhodamine and merocyanine to get new NIR dyes with long absorption and emission (>700 nm) while retaining the rhodamine-like fluorescence ON-OFF switching mechanisms [16]. The Flav [17] and CX [18] series dyes, constructed by linking heterocycles with cyanine dyes, exhibited a 200–300 nm redshift than ICG. However, most of them focused on the fluorescence imaging ability, and the photodynamic or photothermal effects of these dyes were not discussed.

Drug delivery systems can provide the platform for the combination of PTT and/or PDT with other traditional treatments to produce additional or even synergistic therapeutic effects [19,20]. Meanwhile the targeting effect of nanohybrids can improve the delivery of phototherapy agents to tumor tissues, making it possible to improve the selectivity and effectiveness of PTT and PDT. Besides, the combination with other therapies may increase the antitumor effect with low-dose photosensitizers or low-power light irradiation, thereby minimizing potential toxicity to healthy tissues. Among the combinational therapy, the combination of PDT/PTT/chemotherapy may provide synergistic therapeutic effects. Chemotherapy can solve the limitation of light spectrum and can solve the problem of chemotherapeutic drug resistance [21–23].

In this work, based on the fluorophore integration strategy, a novel NIR dye ML880 was designed and synthesized by imitating the cyanine dye polymethine system through a simple synthetic route. The introduction of napththalimide can result in the enhancement of electron delocalization, and the maximum absorption and emission wavelengths of ML880 in DMSO were redshifted to 880 nm and 912 nm, respectively, exhibiting remarkable photothermal effects, compared with the commercial dye IR820. Furthermore, taking advantage of the high level of reactive oxygen species (ROS) in tumor microenvironment, we constructed a photothermal/ROS dual-responsive degradable drug delivery system NBD@ML@RMON for NIR laser and ROS-assisted combination therapy of hepatocellular carcinoma. The ROS responsive mesoporous organosiloxides (RMON) were prepared by H₂O₂-responsive thioacetal fragment organosilane and tetraethoxysilane (TEOS). The NIR dye ML880, and a specific inhibitor of Glutathione-S-transferase NBDHEX [24] were co-sealed into RMON by using phase change materials (PCM) as the thermal responsive gatekeeper. The RMON could be destroyed by intracellular high concentration of H₂O₂ and the ROS produced by ML880. Under 880 nm laser irradiation, the photothermal effect of ML880 melts the PCM, and the loaded cargos will be released, not only realizing PTT/PDT treatment, but also enabling the therapeutic agents’ on-demand release at tumor sites (Scheme 1).

2. Materials and methods

2.1. Materials and apparatus

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. ¹H NMR and ¹³C NMR spectra were measured on a Bruker AMX-400 NMR spectrometer and Bruker Ascend 600 NMR spectrometer, using TMS as an internal standard. High resolution mass spectrometric (HRMS) analyses were carried out in a HP 1100 LC-MS spectrometer. UV–visible spectra and fluorescence spectra were measured on a Horiba Duetta. Deionized water was purified using a Millipore Milli-Q A10 super-water system. The size distribution and zeta potential of nanohybrids were obtained by the Surface Area and Porosimetry System (ASAP2010 N, USA). Fluorescence images were collected on an A1R confocal laser scanning microscope (CLSM, Nikon, Japan).

2.2. Preparation of ML880

ML880 was synthesized through a five-step route (Scheme 1A). The commercial compound 4-bromo-1,8-naphthalene anhydride was first substituted by n-butylamine, obtaining compound ML-1 [25–27]. ML-1 was then reacted with hydrazine hydrate to obtain compound ML-2 [28], followed by Fischer indole synthesis to obtain compound ML-3 [29]. Ammonium salt formation was performed by using ML-3 with 1,3-propanesultone to afford ML-4 [30–32]. The positive charge of

Scheme 1. (A) The synthesis route of NIR fluorophore ML880; (B) The schematic illustration of the preparation and function of nanohybrids.
pyrrolidinium ion of ML-4 makes the α-methyl group acidic thus enabling it to condense on both sides with the aldehydic and vinlydic alcohol of ML-5 to yield the desired product ML880 [33–35]. The structure of ML880 was confirmed by 1H NMR and 13C NMR spectral and ESI-MS. The detailed operational processes were supplied in Supporting Information.

2.6. Cell viability

MTT reagent was used for cell viability analysis. The cells with a good growth state and in the logarithmic growth period were first seeded into 96-well cell culture plates. Each group has 6 multiple wells, and the inoculation density was 7 × 10³/well. Then the culture plates were put into a cell incubator (37°C, 5% CO₂) after cells were adhered to the bottom of each well, the complete medium of each well was discarded and washed twice with fresh DMEM. Subsequently, HepG2 cells were immersed in 100 μL of DMEM containing 10% FBS and 1% penicillin-streptomycin (100 units mL⁻¹ of both) at 37°C under a humidified atmosphere with 5% CO₂.

HepG2 cells were incubated in confocal dishes for 24 h. After replacing the original medium, the HepG2 cells were incubated with a fresh medium containing ML880 or NB6@ML@RMON. Then the cells were incubated with a serum-free medium containing 1 μg mL⁻¹ of Hoechst for 10 min and 10 μM of DCFH-DA for 30 min. After replacing the medium with a fresh medium, dishes were illuminated with or without 880 nm laser. Images were captured using CLSM.

2.7. Animal experiments

Female BALB/c nude mice were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. and used under protocols approved by the Scientific Investigation Board of the East China University of Science and Technology. The volume of tumors was calculated as: Volume = (a × b²)/2, where a represents the longest diameter and b represents the shortest diameter. Six-week-old female BALB/c nude mice with around 100 mm³ HepG2 tumor xenografts were randomly divided into seven groups (n = 5 each). Each group of mice received a different treatment, as follows. Group 1: 100 μL saline; Group 2: 100 μL free NB6@RMON (1.5 mg kg⁻¹); Group 3: 100 μL NB6@RMON (20 mg kg⁻¹); Group 4: 100 μL ML@RMON (20 mg kg⁻¹); Group 5: 100 μL ML@RMON (20 mg kg⁻¹), NIR laser irradiated for 5 min; Group 6: 100 μL NB6@ML@RMON (30 mg kg⁻¹); and Group 7: 100 μL NB6@ML@RMON (30 mg kg⁻¹), NIR laser irradiated for 5 min. The treatment period was 15 days. Each group was injected every three days, and the body weight and tumor sizes were recorded every three days. Groups 5 and 7 were irradiated 18 h after intravenous injection.

3. Results and discussion

3.1. Optical properties of ML880

The absorption and emission features of ML880 were explored in various solvents as shown in Table 1 and Fig. S1. As expected, the maximum absorption and emission wavelengths of ML880 fall in the near-infrared region of 850–940 nm, indicating that the integration strategy to construct new NIR dye based on traditional dye naphthalimide and cyanine was successful. In DMSO, the maximum absorption and emission wavelength of ML880 was 880 nm and 912 nm, respectively. Taking the reported dye ECX [13] as the reference, the fluorescence quantum yield of ML880 in DMSO was 0.11, and the extinction coefficient reached 8.84 × 10⁴ (Table 1). Furthermore, the pH tradition showed that ML880 stayed stable in the range of pH 4–8 (Fig. S2).

To explain the spectral redshift mechanism of ML880, density functional theory (DFT) and time-dependent DFT (TDDFT) calculations were further performed to study the corresponding optimized geometry and electronic structure compared to IR820 as a reference (see Fig. 1). As shown in Table S1, the bond lengths of bonds A-H were listed and the molecular skeleton displayed a symmetrical structure with similar bond lengths for bonds A-D and bonds E-H. The bond lengths of bond A/C in ML880 (1.3929 Å/1.4062 Å) were longer than those of IR820 (1.3916 Å/1.4054 Å). Oppositely, bond B/D in ML880 (1.4046 Å/1.4172 Å) was shorter than those in IR820 (1.4086 Å/1.4178 Å). Compared to IR820, the long bond in ML880 became shorter and short bonds became longer, indicating that ML880 possessed a more delocalized electronic structure. According to the TDDFT results as shown in Table S2, the calculated emission wavelengths at the optimally-tuned TD-PCM(DMSO)-LC-BLYP*/6-311G(d) level well reproduced the experimental measurements, suggesting the reliability of the current level of the method. Moreover, the lowest singlet excited state (S₁) of ML880 and IR820 consisted of a pure LUMO → HOMO transition with significant localized excitation character along the whole molecular backbone.

To further study the fragment’s contributions to HOMO/LUMO orbitals (Table S3), ML880 and IR820 were divided into three fragments (fragment 1 & 3 and fragment 2) as shown in Fig. 1A and D.

| Solvent | λabs [nm] | λem [nm] | ε [M⁻¹ cm⁻¹] | Φ (%) |
|---------|-----------|-----------|---------------|-------|
| PBS     | 936       | NA        | 58000         | NA    |
| DMSO    | 880       | 912       | 88390         | 11.1  |
| MeCN    | 862       | 886       | 36350         | 13.8  |
| DMF     | 868       | 901       | 18600         | 13.1  |
| MeOH    | 856       | 884       | 45890         | 8.2   |
| EtOH    | 862       | 892       | 41720         | 10.1  |

Table 1: Photophysical characterization of ML880 in different solvents.
Interestingly, for HOMOs, both ML880 and IR820 possessed 30% contribution from fragment 1&3 and 70% contribution from fragment 2. However, for LUMOs, the fragment 1&3 in ML880 possess 25% contribution which was significantly larger than that of 12% in IR820, indicating the introduced naphthalimide group played a key role in enhancing the electron delocalization effect. To further quantitatively study the extent of delocalization, the ODI (Orbital delocalization index) coefficient was calculated as shown in Table S3. The smaller the ODI is, the more delocalized the orbitals are. The ODI coefficients of HOMO/LUMO (4.17/4.97) of ML880 were smaller than those of IR820 (4.38/6.08), showing ML880 was more delocalized than IR820. In short, covalent combination between naphthalimide and cyanine can lead to stronger delocalization of electronic structure and larger redshift in emission.

3.2. Photochemical properties of ML880

Since the fluorescence intensity of ML880 in PBS was very weak, we speculated that ML880 had good photodynamic or photothermal effect. Next, the photothermal and photodynamic properties of ML880 were investigated upon 880 nm laser irradiation. Under different concentrations and laser power densities, ML880 displayed remarkable photothermal property. A temperature increase from 36.3 °C to 52.9 °C was recorded with 20 μM aqueous solution of ML880 under 1 W cm⁻² for 8 min (Fig. 2A and B), while only 3.5 °C increase was observed in the PBS. ROS generation was verified by commercial probe DPBF in vitro (Fig. S4) and DCFH-DA in HepG2 cells [36]. As shown in Fig. S4, the absorbance at 410 nm decreases continuously along with the irradiation under 880 nm laser, indicating that ML880 produces singlet oxygen under 880 laser irradiation. The ESR diagram also showed the generation of singlet oxygen when ML880 was treated with laser irradiation. The measured singlet oxygen yield of ML880 is 0.006 (calculated using ICG as reference, of which the singlet oxygen yield in Methol is 0.008) [37]. As shown in Fig. 2C and D, the fluorescence intensity of DCFH-DA was 2.3-fold higher than the control group without laser irradiation, demonstrating that ML880 could produce ROS under 880 nm laser irradiation. Moreover, there was no dark cytotoxicity observed even when high concentration of ML880 was used (100 μM), suggesting the dye had good biocompatibility (Fig. S3). In contrast, the cell viability decreased when increasing the concentration of ML880 upon irradiation with a 0.5 W cm⁻² laser for 5 min, demonstrating that the photothermal and photodynamic effect of the dye in cells could be triggered by NIR irradiation.

3.3. Characteristic analysis of nanohybrids

ROS-responsive mesoporous organosilica nanocarrier (RMON) was designed to co-load ML880 and NBDHEX [38–40] with PCM as the thermal responsive gatekeeper [41], thus constructing nanomedicine NBD&ML@RMON. The nanocarrier could be degraded by the high level of H₂O₂ in the tumor cells, and the ROS produced by ML880 also accelerated the degradation of RMON, exerting the photo controlled release behavior at the tumor site [42–44]. RMON was prepared using H₂O₂-responsive thioacetal fragment organosilane and tetraethoxysilane (Scheme S2). The morphology of RMON was observed by TEM and SEM, which exhibited spherical particles with an average diameter of 91 ± 5.6 nm (Fig. S5). The pore size distribution of RMON showed a peak centered at 2.56 nm, the BET surface area was 972.4 m²/g and the pore volume was 0.62 cm³/g, which was suitable as a drug delivery nanocarrier (Fig. S6). The
The degradability of RMON could be confirmed by dynamic light scattering (DLS; Fig. 3A) and TEM (Fig. S7). As was shown, the degradation fragment size of RMON co-incubated with \( \text{H}_2\text{O}_2 \) is around 5.6 nm, which could be eliminated from the body through renal clearance [45,46]. After being co-loaded with ML880 and NBDHEX and modified with PCM, nanomedicine NBD\&ML@RMON was constructed with an
3.4. Photochemical properties of NBD&ML@RMON

Firstly, the photothermic property of NBD&ML@RMON was probed in the PBS solution. Upon NIR laser irradiation (880 nm, 1 W cm\(^{-2}\)), photothermal images were recorded longitudinally. The NBD&ML@RMON dispersion dramatically increased the solution temperature. The temperature increase of NBD&ML@RMON (\(\Delta T = 30^\circ C\)) NPs was much higher than that of PBS (\(\Delta T = 3^\circ C\)) (Fig. 3D). The significant temperature increase clearly demonstrated the ultra efficient photothermal property of NBD&ML@RMON. Then the photothermal conversion efficiencies (\(\eta\), PTCE) were also measured by following a reported method [47]. A steady-state temperature was recorded through the heating and cooling process and the PTCE was calculated to be 65.4% (Fig. 5E and F). As shown in Fig. S10, NBD&ML@RMON exhibited outstanding thermal stability and photostability, showing no degradation after four cycles of heating and cooling under continuous 880 nm laser irradiation. Meanwhile, the photodynamic property of NBD&ML@RMON was also evaluated in HepG2 cells. The fluorescence intensity of DCFH-DA was 3-fold higher than the control group without laser irradiation, demonstrating that NBD&ML@RMON could produce ROS under 880 nm laser irradiation (Fig. S11).

Then the property of NIR light-controlled release of NBD&ML@RMON was investigated under intermittent 880 nm laser irradiation. In Fig. S12, after being placed in a constant temperature water bath (37 \(^\circ\)C) after 30 min, NBD&ML@RMON release amount was only 24.3% without laser irradiation. In contrast, in the case of 880 nm laser intermittent irradiation, the total amount release of NBDHEX was gradually increased. At the time point of 17 min, the cumulative release rate of NBDHEX was 66.5%. In the absence of light, the cumulative release of NBDHEX was negligible. The incubation time prolonged to 3 h was also tested (Fig. S12b). The drug release gradually stabilized after incubation for 2 h. The cumulative drug release of NBDHEX in 3 h was 26.1%. Meanwhile the cumulative drug release of NBD&ML@RMON under 42 \(^\circ\)C water bath was 78.2%. These results indicated that approximately 52% of the NBDHEX was released through thermal response. Moreover, drug release curve of NBD&ML@RMON under different \(H_2O_2\) concentrations was also recorded (Fig. S12b). The cumulative drug release of NBD&ML@RMON after 3 h was 27.4%, 39.1% and 51.7% when \(H_2O_2\) concentration was 1 \(\mu\)M, 100 \(\mu\)M and 1 mM, respectively. Cellular uptake of NBD&ML@RMON was assessed through confocal microscopy. The fluorescence intensity of cells cultured for 24h was 1.8-folder higher than that of cells cultured for 12h, which indicated the cell uptake of the NBD&ML@RMON (Fig. S13).

Photo-controlled release of the drug was also examined in HepG2 cells. The fluorescence intensity of NBDHEX with laser irradiation was 1.5-fold brighter than the control group without laser. The PCM was melted by the photothermal effect produced by ML880, which caused the drug NBDHEX to be released from NBD&ML@RMON into the cells, revealing that NIR laser irradiation accelerated the release of the drug from nanohybrid (Fig. S14). The above data fully confirmed the good ability of the NIR light-controlled release drug of nanohybrid NBD&ML@RMON.

![Fig. 4. Cell viability of HepG2 cells incubated with NBD@RMON, ML@RMON and NBD&ML@RMON at various concentrations (A) after NIR light irradiation (880 nm, 1 W cm\(^{-2}\), 5 min) and (B) in the dark. (C) Dark cytotoxicity of L929 cells incubated with NPs. (D) CLSM images of HepG2 cells subjected to different treatments according to the live and dead cell assays (green fluorescence, calcein AM staining living cells; and red fluorescence, PI staining dead cells; scale bar = 100 \(\mu\)m).](image-url)
3.5. Cytotoxicity of nanohybrids in cells

The phototoxicity of NPs to HepG2, L929 and Lo2 cells was examined via MTT assay. As shown in Fig. 4A, dose-dependent cytotoxicity of NBD@RMON against the HepG2 was observed after the cells were irradiated by 880 nm laser, while neither NBD@RMON nor ML@RMON exhibited significant cytotoxicity. Nearly no cytotoxicity was observed after the HepG2 cells were treated with 160 μg/mL nanohybrids in the dark (Fig. 4B). The cytotoxicity of nanohybrids towards normal cells was also investigated. All of the nanohybrids showed negligible cytotoxicity in L929 cells (Fig. 4C) and Lo2 cells (Fig. S15), suggesting that NBD@RMON had good biocompatibility. In the flow cytometry-based apoptotic cells analysis of HepG2 cells, the apoptosis rate detected using Annexin V-FITC/Propidium Iodide (PI) staining is less than 1% after treated with PBS, NBD@RMON, whether under irradiation or not. After treatment with ML@RMON, 11.9% of cell apoptosis is recorded under irradiation while only 0.6% in the absence of laser. After being treated with NBD@RMON, 92.6% and 0.6% of cell apoptosis was recorded with and without irradiation respectively, which is consistent with the results of cytotoxicity experiments (Fig. S16). These results demonstrated that the over-express H2O2 in tumor cells and the increased temperature and ROS produced by NBDHEX & ML@RMON could be control-released, realizing the co-loading of chemotherapy drug NBDHEX could be control-released, realizing the co-loading of chemotherapy drug NBDHEX and ML@RMON under NIR laser irradiation, no obvious temperature changes in the tumor site were observed in the group treated with NBDHEX. After tail vein injection with NBD@RMON, the highest temperature could reach 45.7 °C at 18 h post injection while fall to 43.6 °C at 24 h post injection, which means the maximum accumulation of NBD&ML@RMON in the tumor region was around 18 h post injection. These results also showed the prominent enhanced permeability and retention effect (EPR) and the photothermal effect of the nanohybrids (Fig. 5A). Then the mice were sacrificed and harvested the main organs and tumors to analyze the tissue distribution of NBDHEX. The experiments revealed that the highest concentration of NBDHEX (6.51 ng/mg) was detected in the tumor region of the mice treated with NBD&ML@RMON (Fig. 5B), while only 1.74 ng/mg of NBDHEX was detected in the group treated with free NBDHEX, indicating high tumor accumulation of NBD&ML@RMON.

Antitumor potency of the nanohybrids was investigated using HepG2 tumor-bearing Balb/c female nude mice (Fig. 6A). Mice were divided into seven groups randomly, as follows: Group 1: Saline; Group 2: Free NBDHEX; Group 3: NBD@RMON; Group 4: ML@RMON; Group 5: ML@RMON + laser; Group 6: NBD&ML@RMON, Group 7: NBD&ML@RMON + laser. As shown in Fig. 6B, the tumor growth in group 5 was evidently suppressed, suggesting that the PTT and PDT induced by ML880 under NIR laser irradiation could inhibit cancer effectively. Significantly, a better tumor eradication compared with other groups was observed in group 7, indicating that cooperative interactions between PTT/PDT/chemotherapy could potentially increase the antitumor efficacy. The weights of all the mice in seven groups slowly increased, indicating that the treatments did not cause systemic toxic effects on mice (Fig. 6C). After 15-day therapy, mice were then sacrificed and harvested the main organs and tumors. The ananotized tumor displayed eradication and the lowest values in size and weight in group 7 than those in the other six groups (Fig. 6D and E). It was worth noting that the tumor growth inhibition rate of Group 5 and Group 7 reached 84.3% and 94.3%, respectively (Fig. 6F). The phototherapeutic effect produced by ML880 in Group 5 was effective to inhibit the tumor growth, while combination with chemotherapy in Group 7 produced a remarkable tumor suppression even eradication. The bioactivity of residual tumor tissues was further verified through a pathological examination by hematoxylin and eosin (H&E) staining and TUNEL experiments. The H&E staining and TUNEL of tumor slices indicated that Group 5 and 7 exerts plenty of tumor cells apoptosis (Fig. 6G and H). No obvious histopathological abnormality was observed from normal organs among seven groups, verifying the excellent biocompatibility of nanohybrids (Fig. S17). The pharmacokinetic profiles of the free NBDHEX and NBD&ML@RMON were further studied in Sprague-Dawley (SD) rats by determining the fluorescence intensity of NBDHEX in plasma. As shown in Fig. S18, the incorporation of NBDHEX in nanohybrids enables higher NBDHEX concentrations in the blood circulation than free NBDHEX over 48 h. The maximum plasma concentration of NBDHEX in mice injected with NBD&ML@RMON was 7.38 μg/mL, much higher than that of free NBDHEX, at 4.96 μg/mL. There was a significant 5.6-fold improvement in the AUC (Area Under Curve) between the NBD&ML@RMON (139.42 mg/L h) and free NBDHEX (24.65 mg/L h), indicating that NBD&ML@RMON could

![Fig. 5. (A) Photothermal imaging of the tumor region under 880 nm laser irradiation (1 W cm⁻², 5 min). (B) In vivo drug distribution in mice after 24 h with free NBDHEX and NBD&ML@RMON.](image)
improve the bioavailability of NBDHEX after systematic administration.

Furthermore, immunofluorescence staining images showed that NBDHEX could inhibit GSTP1-1 effectively (Fig. S19). We further monitored the expression of apoptosis-associated proteins [51]. The mitochondrial apoptotic pathway (a major signalling way to involve in tumor cells apoptosis) associated proteins of caspase-3, Bax (pro-apoptotic protein), and Bcl-2 (antiapoptotic protein) in tumor cells were detected by Western blot, and the apoptosis-related proteins levels were shown in Fig. 7. It was found that the Bcl-2 level of Group 5/7 was obviously down-regulated, while the pro-apoptotic protein Bax was significantly up-regulated compared to other groups. Moreover, the downstream protein active-caspase 3 was highly expressed in group 5/7, which could be ascribed to massive cell apoptosis. These results showed that the HepG2 cells in Group 5 and 7 treated with ML@RMON and NBD&ML@RMON respectively were apoptosis by mitochondrial apoptosis pathway under NIR laser (880 nm) light.

4. Conclusion

In summary, a new NIR dye ML880 with naphthimide modified
cyanine skeleton was designed and synthesized using an integration strategy. By introducing the naphthalimide moiety, ML880 exhibited stronger electron delocalization and larger redshift in emission compared with IR820. Then ML880 was encapsulated with the chemotherapy drug into photothermal/ROS dual-responsive RMON to construct nanomedicine NBDA&ML@RMON. Both in vitro and in vivo experiments demonstrated excellent therapeutic effects of NBDA&ML@RMON, implying that combined PDT/PTT/chemotherapy could remarkably inhibit tumor growth with high biosafety under 880 nm laser irradiation. Our study is a successful attempt to prolong the wavelength of traditional dyes by integration strategy and open a new platform for the future design of phototherapeutic agents by combination with various therapies in comprehensive cancer treatment.

Declaration of interests

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

CRediT authorship contribution statement

Tongxia Jin: Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Writing – original draft. Di Cheng: Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Writing – original draft. Guanyu Jiang: Methodology, Data curation, Formal analysis, Investigation, Writing – original draft. Wenqian Xing: Investigation. Peiwen Liu: Investigation. Bin Wang: Investigation. Weiping Zhu: Conceptualization, Methodology, Data curation, Formal analysis, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. Haitao Sun: Methodology, Data curation, Formal analysis, Writing – review & editing, Funding acquisition. Zhennong Sun: Supervision. Yufang Xu: Supervision. Xuhong Qian: Resources, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioactmat.2021.12.009.
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