Recent advances of transition Ir(III) complexes as photosensitizers for improved photodynamic therapy

Liping Zhang¹,² | Dan Ding¹

¹ State Key Laboratory of Medicinal Chemical Biology, Key Laboratory of Bioactive Materials, Ministry of Education, and College of Life Sciences, Nankai University, Tianjin, P. R. China
² Shenzhen Key Laboratory of Neurosurgery, Shenzhen Second People’s Hospital, Shenzhen, P. R. China

Correspondence
Dan Ding, State Key Laboratory of Medicinal Chemical Biology, Key Laboratory of Bioactive Materials, Ministry of Education, and College of Life Sciences, Nankai University, Tianjin 300071, P. R. China.
Email: dingd@nankai.edu.cn

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Abstract
Photodynamic therapy (PDT), a minimally invasive procedure, usually required photosensitizer (PS) under light irradiation to convert the absorbed light energy into reactive oxygen species (ROS) to cause cancer cell apoptosis. The PSs with abundant excited triplet state play a critical role to guarantee the PDT effect. Transition Ir(III) complexes has been proved to be an effective PSs for PDT, owing to their high intersystem crossing (ISC) ability, tunable optical properties, long excited state lifetimes. However, poor biocompatibility, short excitation wavelength, and high oxygen dependence limit their biomedical applications. This review summarizes important progress of Ir(III) complexes as PS in improved PDT, including: (1) the strategy of nanoparticles is employed to improve their biocompatibility, and significantly enhance the cellular intracellular efficiency at the same time. (2) Their excitation wavelength is successfully extended by combining fluorophores, forming nanoscale Metal-Organic frameworks (nMOFs) or two-photon excited therapy. (3) Their therapeutic effect for antihypoxic tumors is enhanced by constructing mitochondrial-targeted PS or type I PDT. In addition, simple modification of the precursor is beneficial to obtain a more excellent therapeutic effect. Finally, we briefly summarize the current challenges and future research opportunities for Ir(III) complexes in this research field.

KEYWORDS
biocompatibility, deep tissue penetration, hypoxia, Ir(III) complexes, photodynamic therapy

1 | INTRODUCTION

At present, photodynamic therapy (PDT) has widely considered to be an effective technique for cancer treatment, due to the advantages of noninvasiveness, safety, and high selectivity compared with traditional chemotherapy, surgery, and radiotherapy.¹⁻⁴ In general, the cancer cell apoptosis caused by PDT go through two steps: (1) generating an excited triplet state (PS*) via the photosensitizer (PS) under light irradiation; (2) converting the absorbed light energy of PS* to highly cytotoxic reactive oxygen species (ROS).⁵,⁶ As shown in Figure 1, the generated ROS can be divided into two categories, including hydrogen peroxide (H₂O₂), hydroxide (OH⁻), and superoxide anion.
FIGURE 1 Schematic diagram of the photophysical and PDT

(O$_2^-$), produced by type I PDT, and single oxygen (^{1}O$_2$) generated by type II PDT.$^{7,8}$ In the last few years, a number of organic agents based on organic fluorescent small molecules dyes, such as boron dipyrromethene (BODIPYs) or porphyrin, and their derivatives have been reported to apply in PDT.$^{7,10}$ Their treatment effect does not conform to the clinical standards, owing to insufficient generating PS* capacity. Therefore, PSs with abundant excited triplet state plays a critical role to guarantee their PDT effect.

One of available strategy to enhance the efficiency of PS* production is to increase the intersystem crossing (ISC) through introducing the heavy atoms into PSs such as halogens, transition metals, etc.$^{11–13}$ Ir(III) complexes are receiving considerable attention as potential PSs for PDT at present, because of their longer excited lifetime, high ISC ability, and good photothermal stability.$^{14–16}$ However, the traditional Ir(III) complexes PSs are far from ideal and exhibit several challenges: (i) the poor water solubility and serious dark toxicity seriously hinders their application in biology.$^{10,17,18}$ (ii) The inherently short excitation wavelength results in the poor tissue penetration and severe photodamage, becoming a key obstacle in clinical application.$^{19–21}$ (iii) their therapeutic effect is highly subject to the oxygen content in the tumor which seriously limits their efficacy in PDT.$^{22–24}$ So, not surprisingly, the Ir(III) complexes system attracts attention is less than small organic molecule. Nonetheless, important progress has been made with Ir(III) complexes in PDT. These studies clearly reveal that Ir(III) complexes are feasible in practical applications, and simple modification of the precursors will accelerate the development of more efficient materials.

This review article presents the important progress of Ir(III) complexes as PS in improved PDT, and highlights the latest strategies for Ir(III) complexes to solve biocompatibility, tissue penetration, and hypoxia. The therapeutic effect can be rapid improved by simple modifying the precursor. It is our purpose to inspire more researchers to enter this captivating field.

2 | IMPROVE BIOCOMPATIBILITY STRATEGIES

The excellent biocompatibility of PSs plays a vital role in ensuring their effective therapeutic effects.$^{25–27}$ Traditional Ir(III) complexes are generally hydrophobic in aqueous media because of their rigid structures and strong π-π stacking effect, resulting in the poor internalization efficiency and reduced ROS generation.$^{28,29}$ In addition, their non-negligible cytotoxicity is still a controversial issue, which severely limits their clinical applications.$^{30}$ For this reason, the development of Ir(III) complexes with good water solubility and negligible dark toxicity is of great significance to promote their progress in biological research and biomedical applications.

Fortunately, the strategy of nanoparticles (NPs) has successfully solved the above problems.$^{31,32}$ Nanomaterials are attracting growing attention in the biological field because they have many outstanding advantages: (i) the hydrophilic shell can enhance their solubility in the physiological environment and reduce the dark toxicity.$^{33}$ (ii) The surface of NPs can be easily modified by recognition groups to enhance their targeting target function.$^{34,35}$ (iii) NPs can passively accumulate in tumor sites and extend the plasma residence time via enhanced permeability and retention effect.$^{36,37}$ (iv) The emission intensity and ^{1}O$_2$ generation ability of NPs obviously improved are beneficial for imaging and therapy.$^{38,39}$ So far, many effective strategies have been developed for Ir(III) complexes to construct the NPs such as polymer-conjugation technique, nanoprecipitation, self-assembly method, and introducing hydrophilic ions.$^{30,40–42}$ This section will emphasize on
2.1 Ir(III)-PS nanoparticles prepared by polymer-conjugation technique

In consideration of the strongly delocalized π-conjugated backbones, the conjugated polymer exhibits high molar absorption coefficient, bright emission and good photosensitivity, which has become a promising photosensitive material in the biological field. A series of conjugated polymer nanoparticles with good biocompatibility are emerging rapidly, and are developing into promising photosensitizers for photodynamic therapy (PDT).

In 2010, Ir(III) polypyridine poly(ethylene glycol) (PEG) complexes were designed and synthesized for the first time by Lo and coworkers. The half maximal inhibitory concentration (IC50) of the obtained water-soluble Ir(III)-PEG complexes was 90 times higher than that of the corresponding PEG-free Ir(III) complexes, indicating that their biocompatibility was significantly improved. In addition, the introduced water-soluble PEG polymer chain is significantly beneficial for enriching photophysical properties, improving water solubility, reducing cytotoxicity, and enhancing cellular uptake efficiency, which can be attributed to restraint the interaction between the complex and intracellular DNA, proteins and organelles.

Subsequent, Huang and coworkers constructed two semiconducting polymer dots (Pdots) (WPF-Ir8 and WPF-Ir4) by introducing different ratios of Ir(III) complexes and polyfluorene units into the main polymer chains as PSs for PDT (Figure 2A). In this system, the obtained Pdots possess ultrasmall particle size (6 nm), outstanding biocompatibility, and can effectively transfer energy from the polymer backbone to Ir(III) complexes (Figure 2B). Interestingly, they can be employed as optical probes to high sensitivity monitor oxygen in aqueous solutions (Figure 2C). After irradiation, the apoptosis rate of HeLa cells treated with Pdots reached 80%, implying high-performance phototoxicity (Figure 2E). Recently, Huang’s research group further developed a water-soluble conjugated polymer-Ir(III) complexes based on the glycopolymers polygalactose (PGal). This brush polymer can successfully target the Hep G2 cells, because that the PGal can specifically recognize asialoglyco-protein receptor (ASGPR) overexpressed in Hep G2 cells. Under the irradiation of white light, the achieved PSs can effectively inhibit the growth of xenograft Hep G2 tumor.

2.2 Ir(III)-PS nanoparticles prepared by nanoprecipitation

Nanoprecipitation, a general method, relies on hydrophobic organic PS encapsulated by amphiphilic polymers to form NPs with a typical core-shell structure. Comparing with polymer-conjugation technique, nanoprecipitation tends to be more universal and amenity because it does not require further adjustment of its chemical structure. The size of NPs can be fine tuned by changing the ratio of organic PS and amphiphilic polymer, thereby control accumulation and distribution of PSs in vivo.
However, the Nanoprecipitation strategy is not suited for conventional PSs owing to aggregation-caused quenching (ACQ) caused by reduced emission intensity and ROS generation ability. In 2001, a novel type of PSs with aggregation-induced emission (AIE) was successfully discovered by Tang’s group to solve this problem, by restriction of intramolecular motions which restrain the dissipation of energy. AIE NPs prepared by nanoprecipitation have been widely employed as PSs for PDT. However, most present reposts place emphasis on small organic molecules, rather than Ir(III) complexes, maybe causing by the difficulty of synthesizing AIE Ir(III) complexes.

Recently, TPA is employed as a bridge to synthesize a series of deep red-emitting AIE Ir(III) complexes with different number of Ir centers (mono-, di-, and trinuclear) by our group (Figure 3A). The corresponding NPs were obtained by nanoprecipitation, comprising the hydrophobic Ir(III) complexes as the core, the hydrophilic 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[maleimide(poly(ethylene glycol))-2000] (DSPE-PEG-MAL) as the shell, and the HIV-1 transactivator (RKKR-RQRRRC) as the surface functionalization group (Figure 3B). All of them possess high drug loading efficiency (80%), uniform spherical morphology, suitable size (80 nm), and outstanding stability. Compared with the pure Ir(III) complexes PS1, PS2, and PS3, the corresponding NPs are more satisfying for brighter emission, higher phosphorescence quantum yields (35%), longer excited lifetime (4.61 μs), higher $^{1}$O$_{2}$ generation ability, better biocompatibility, and superior cellular uptake. Furthermore, the effect of PDT increases with the increase in the number of Ir centers (Figure 3E). Importantly, the molar absorption coefficient increases as the number of metal centers for the Ir(III) complexes and their NPs with the decreasing order: $\text{PS1} (\varepsilon = 7660 \text{ m}^{-1} \text{ cm}^{-1}) < \text{PS2} (\varepsilon = 16 176 \text{ m}^{-1} \text{ cm}^{-1}) < \text{PS1 NPs} (\varepsilon = 17 570 \text{ m}^{-1} \text{ cm}^{-1}) < \text{PS3} (\varepsilon = 31 143 \text{ m}^{-1} \text{ cm}^{-1}) < \text{PS2 NPs} (\varepsilon = 43 651 \text{ m}^{-1} \text{ cm}^{-1}) < \text{PS3 NPs} (\varepsilon = 72 935 \text{ m}^{-1} \text{ cm}^{-1})$. The absorbance of PS1 NPs and PS3 are 2.29 and 4.07 times higher than PS1, respectively. High molar absorption coefficient means a high PDT effect. Upon irradiation, tail vein injection of PS3 NPs with the topmost PDT performance can efficiently inhibit tumor growth in vivo (Figure 3F).

### 2.3 | Ir(III)-PS nanoparticles prepared by self-assembly method

NPs prepared by introducing biocompatible polymers as PSs have made remarkable achievements in the biological field. However, these reported systems have drawn some scepticism due to the presence of multiple components with potential biological toxicity, which limits their practical clinical applications. Recently, it has been...
proved that organic molecules can self-assembled form pure, single-component, and good biocompatibility NPs to overcome the above problems.

In 2017, Xie et al. reported a cyclometalated Ir(III) complex (IrBDP) bearing boron-dipyrromethene (BDP) units, and the corresponding pure NPs formed via self-assembly (Figure 4A).\textsuperscript{69} The hydrophilicity and molar absorbance at long wavelength of IrBDP are improved by introducing BDP units, further promoting its drug loading efficiency and PDT effect. Transmission electron microscopy (TEM) and dynamic light scattering (DLS) showed that the obtained IrBDP NPs exhibit spherical morphology and uniform sizes (190.2 nm). The bright red emission was observed from HeLa cells incubated with IrBDP NPs, confirming that the self-assembled NPs can be effectively internalized. The cell viability of CT26 and HeLa treated with IrBDP NPs were still higher than 95%, obviously splendid biocompatibility. In contrast, under light irradiation (16 mW/cm\textsuperscript{2}, 530 nm, 30 min), the cell viability was obviously reduced, implying excellent potent phototoxicity.

More recently, our group reported for the first time that pure NIR-emitting AIE self-assembled multinuclear Ir(III) complex NPs was used as PS in PDT (Figure 4B).\textsuperscript{42} The flexible imine ligands are employed to construct AIE molecules. The rigid 1,3,5-triphenyl benzene can effectively extend the \( \pi \)-conjugated skeleton, which is beneficial for obtaining near-infrared (NIR) emission. 3-Ir with three positive charges, large \( \pi \)-conjugated, and highly symmetrical structure facilitate it easily to form self-assembled NPs. The acquired spherical and small size (<90 nm) NPs can exist stably in water for over a week, indicating good stability. Notably, the PL intensities and \( ^1O_2 \) generation ability of NPs is obviously higher than their corresponding Ir(III) complexes. In particular, trinuclear 3-Ir NPs with NIR emission (730 nm), outstanding \( ^1O_2 \) generation ability, satisfactory biocompatibility, and remarkable phototoxicity, can effectively kill cancer cells after irradiate.

2.4 | Ir(III)-PS nanoparticles prepared by introducing hydrophilic ionic

In general, on account of their adverse hydrophobicity and ACQ nature to the imaging and PDT effects, most of reported NPs strategies are still unsuitable for traditional Ir(III) complexes.\textsuperscript{70} Currently, ionic groups have attracted great interest in the biological field due to their unique water solubility, satisfying stability, low dark toxicity, and high cellular internalization under physiological conditions.\textsuperscript{71,72} The preparation method of NPs by one-step ion exchange is simple, feasible, and and give high yield. Importantly, ACQ NPs can simultaneously successfully improve their emission intensity, ROS generation efficiency, and biocompatibility for PDT by introducing hydrophilic ions.

In 2019, a series of water solubility Ir(III) complexes containing quaternary ammonium (QA) salts were successfully developed by Hou’s group.\textsuperscript{73} The presence of the QA groups prompts the complexes to overcome the shortcomings of emission quenching and reduced \( ^1O_2 \) generation in aqueous media. A negligible change was found
in the size of the obtained vesicle NPs within 2 weeks due to the strong electrostatic repulsion. The $^3\text{MLCT}$ excited state of NPs is protected by these aggregated vesicles, thereby limiting the energy dissipation of other nonradiative. Meanwhile, the cellular internalization of these water solubility Ir(III) complexes is significantly improved, because the negatively charged QA groups are obviously beneficial to the binding affinity of the complexes with cell membranes. These NPs can be used as satisfactory mitochondrial targeting agents for imaging and inhibits the growth of the tumor cells.

More recently, two new water-soluble Ir(III) sodium salt complexes NPs were reported by our group (Figure 5A)\textsuperscript{41}. These were the first examples of sodium salt Ir(III) complexes as PSs for PDT. The pure NPs with high yields is obtained by a one-step ion exchange reaction of the Ir(III) carboxylic acid complexes with NaOH in water. The sizes of Ir(III) carboxylic acid complexes (1-H: 359 nm; 2-H: 392 nm) are significantly larger than the corresponding sodium salt pure NPs (1-Na: 76 nm; 2-Na: 81 nm), which proves that the incorporation of water-soluble sodium salt can greatly improve water solubility (Figure 5B). Such small size of NPs is conducive to the cell internalization. Compared with the 1-H (617 nm) and 2-H (613 nm), the corresponding 1-Na (660 nm) and 2-Na (643 nm) exhibit an obviously redshift (43 and 30 nm, respectively) and with higher emission intensity. Especially, the PL intensity of 2-Na is 13.8 times larger than that of 2-H (Figure 5C), indicating that the annoying ACQ effect has been perfect overcome. Under irradiation, the IC$_{50}$ values of 1-Na and 2-Na are about 5.9- and 9.6-times lower than those of 1-H and 2-H, respectively (Figure 5D), fully confirming sodium salt pure NPs with excellent phototoxicity.

3 | DEEP PDT STRATEGIES

A good PS must possess strong absorption of visible light, effective ISC, high ROS yield, and deep tissue penetration.\textsuperscript{74,75} The long-wavelength excitation of PSs is crucial to ensure effective therapeutic effects, due to its minimal light damage to normal tissues, satisfactory tissue penetration, and excellent spatial resolution.\textsuperscript{76–78} Unfortunately, the vast majority of Ir(III) complexes are usually triggered by UV or blue light, resulting in ineffectiveness against deep tumors, which is a key obstacle to their practical clinical application.\textsuperscript{21,79} Therefore, it is highly desirable to develop Ir(III) complexes with long-wavelength excitation as PSs for deep PDT treatment.

To address this problem, considerable efforts have been performed to extend the phototherapeutic window of Ir(III) complexes, including (1) combining fluorophores; (2) forming nanoscale Metal–Organic frameworks (nMOFs), and (3) two-photon excited therapy.\textsuperscript{15,80–82} These strategies retain the advantages of Ir(III) complexes with excellent ISC ability, and extend their excitation wavelength, thus, having convincing anticancer activity. In this section, the current strategies for extending the excitation wavelength of Ir(III) complexes will be summarized.
3.1 Ir(III)–Fluorophores combined agents

Organic fluorophores have become an indispensable daily tool in diagnoses and therapy, due to their rich photophysical properties and strongly $\pi-\pi^*$ transitions at long wavelengths. However, their low ROS generation ability is affected by the poor ISC ability, resulting in the dissatisfactory therapeutic effect for PDT. An effective way to improve the ISC ability of PS via introducing transition metal atoms, which effectively enhances the separation of HOMO and LUMO. Through this strategy, the achieved metal-fluorophores combined PSs can obviously merges the advantages of transition metal complexes with the long-lived triplet metal-ligand charge transfer (3MLCT) states and fluorescent molecular strongly $\pi-\pi^*$ transitions at long wavelengths, thus, possessing high ROS generation ability and long-wavelength excitation.

In 2019, Yuksel and coworkers reported a red-wavelength excitation Ir(III) complexes containing two BODIPY units (Figure 6A). Compared with the free BODIPY, the corresponding Ir(III) complexes exhibit similar absorption bands but with higher the molar extinction coefficient (Figure 6B). The $^{1}O_2$ quantum yields ($\phi_\Delta$) of Ir(III) complexes (0.06) was six times higher than free BODIPY (0.01). MTT experiment confirmed that Ir(III) complexes can slightly induce the cancer cells apoptosis under the red irradiation (Figure 6C). To some extent, the synthesized Ir(III) complex can be excited by red light and produce the efficiency of phototoxicity. However, the efficacy is not ideal, which is ascribed to the unmatched distance between the coordination center and BODIPY unit.

In the same year, Novohradsky et al. reported a novel Ir(III)–COUPY combined agents as PS for PDT (Figure 6D). This is the first example of the coupling of a Ir(III) complexes with the coumarin-based COUPY fluorophore. The UV absorption spectra of Ir(III)–COUPY complexes revealed an intense absorption bands around 500-600 nm, which was assigned to the spin-allowed ($\pi-\pi^*$) transitions of the coumarin (Figure 6E). The fluorescence of coumarin can also be scanned when Ir(III)–COUPY was selectively excited, showing effective energy transfer from Ir(III) complexes to coumarin fluorophores. Interestingly, the internalization pathway of Ir(III)–COUPY complex is different from the two separate parts, and is an energy-independent uptake mechanism. Under the irradiation of green light, Ir(III)–COUPY complexes show excellent phototoxicity. These phenomena confirm that combining coumarin into Ir(III) complex can extend excitation wavelength of PSs.
To the most present, Gou’s groups used donor-acceptor-donor fluorescent chromophores with NIR absorption to construct Ir(III) complex, which possesses high ROS generation efficiency, negligible dark toxicity, and high PDT, and PTT effect under 808 nm irradiation. This complex has four advantages: (1) it can self-assemble metallosupramolecular aggregates with remarkable red-shifted absorption in aqueous media, increasing the light penetration depth in biological tissues; (2) it produces a distinct aggregation-induced PDT behavior; (3) it can produce type I PDT for less oxygen-dependent degree; (4) it exhibits high photostability and efficient PDT and PTT synergistic effects under 808 nm irradiation. Compared with conventional Ir-based PSs, this complex exhibits deep NIR light tissue penetration, highly efficient ROS and heat generation, and high photothermal conversion efficiency. As a result, this Ir(III) complex preferentially accumulate in tumor areas and exhibits a high in vivo tumor regression of 96%.

3.2 | Nanoscale metal-organic frameworks base on Ir(III) complex

MOFs, a type of emerging crystalline, are formed by connecting metal ions (or clusters) and organic ligands, which have achieved a remarkable development in the past 20 years, owing to their tailorable pore size, structural flexibility, and large specific surface areas. Especially, nMOFs with high drug loading capacity, good biodegradability, excellent cellular internalization, and long-wavelength excitation, is successfully employed as PSs for biological applications.

In 2017, Lin et al. first reported achieved nMOFs based on Ir(III) Complex as PS for deep PDT (Figure 7A). Upon X-ray irradiation, Hf atoms in the MOFs effectively absorb X-rays and rapidly transfer energy to the Ir(III) complex in the ligands via inelastic scattering to generate $^1\text{O}_2$. The thickness of the obtained Hf-BPY-Ir nMOFs is 1.2 nm, which was conducive to cellular internalization and the rapid diffusion of $^1\text{O}_2$ (Figure 7B). In the MTT experiment, after incubation of CT26 and MC38 cells with Hf-BPY-Ir nMOFs (0–100 μg/mL) for 24 h, the cell viability was still higher than 95%, indicating the good cytocompatibility and negligible dark cytotoxicity. Furthermore, during X-ray irradiation, negligible change in the phototoxicity of Hf-BPY-Ir was observed when the cells were covered with a beef block of 1 cm in thickness, supporting deep tissue penetration.

3.3 | Two-photon excited therapy

Two-photon excited (TPE) is an emerging method that absorbs two NIR photons as an excitation source to access a given excited state of the corresponding one-photon transition, which indicates that the PS absorbed in the UV/Vis region can be activated by NIR light, thereby, enabling deeper PDT. In addition, the damage area caused by TPE is much lower than that of traditional one-photon, because TPE is only triggered by high illumination intensity with a small volume. In general, TPE PDT is one
of the promising methods to develop long-wave excited Ir(III) complexes to achieve deeper penetration depth.

In 2017, a series of TPE AIE Ir(III) complexes was first studied by Chao and coworkers (Figure 8A).95 With the increase of water content, the PL intensity of these compounds increases significantly, confirming the typical AIE effect. DLS experiments show that in a 90% water-DMSO mixture, these complexes formed NPs with a size of 88.99-250.09 nm. Remarkably, Ir1 was observed to possess a high TPA cross-sections (s2) of 214 GM at 730 nm. The uptake and localization experiment confirmed that the obtained these AIE active complexes can selectively accumulate in mitochondria, and the uptake rate measured in cancer cells is higher than that in normal cell lines due to the higher membrane potential. Compared with conventional one-photon PDT, TPA-PDT of Ir1 exhibited lethal more excellent damage to MCTS, which fully proves that the TPA-PDT is more effective for deep tissue tumors.

Recently, Gao et al. reported four mitochondrial-targeted Ir(III) complexes as PSs for TPE PDT (Figure 8B).96 The 1O2 generation quantum yields of Ir(III) complexes is related to their luminous efficiency and the electronic effect of the substituent, and the former plays a decisive role. Ir3 with strongly electron-donating substituent (−OCH3) and luminous efficiency (0.18) exhibits the highest 1O2 generation quantum yields. All compounds showed negligible dark cytotoxicity under dark conditions (IC50 > 160 μg/mL), proving good biocompatibility. Obviously, under light, the IC50 value of Ir3 (0.96 μg/mL) by 800 nm light is lower than that of by 400 nm light (1.35 μg/mL). Encouragingly, in the same condition, the superiority in phototoxic properties of Ir(III) complex irradiated by 800 nm light to those irradiated by 450 nm light is attributed to the excellent penetration of infrared light.

3.4 | Upconversion nanoparticles of Ir(III) complexes for deep PDT

In consideration of the weak absorption of Ir(III) complexes in the NIR region that hinders their practical application to tumor tissue in depth, lanthanide-doped upconversion NPs are used to convert the NIR light to higher-energy visible light. Based on this idea, Ir(III) complexes can be covalently conjugated with the upconversion NPs to realize the NIR light-triggered PDT. For example, Gou’s groups designed a long-lived triplet excited state Ir(III)-naphthalimide complex by conjugating with upconversion-based NPs (Figure 9A).97 In this work, an Ir(III)-based PS with a long-lived intraligand excited state has been first synthesized and shows significantly enhanced singlet oxygen generation efficiency with 45-fold when compared to the conventional Ir(III) complex. To enable deep tissue penetration, this Ir(III) complex was further covalently bonded to the upconversion NPs. When annealing upon NIR irradiation, the complex exhibits efficient conversion of oxygen to 1O2 during the PDT and enhances the PDT effect of tumor tissue. Excitingly, mice with intravenous injection of this complex plus 980 nm irradiations shows significant suppression of tumor growth. Forwardly, Zhou’s groups designed an Ir(III) complex-based polymeric micelle system, which shows low dark toxicity and strong NIR excitation for phototherapy and chemotherapy (Figure 9B).67 This complex containing amphiphilic block polymer is self-assembled and serves as the skeleton. In order to permit NIR excitation, the upconversion NPs are bonded in the polymeric micelles. Compared with the nonformulated Ir(III) complex, this complex exhibits high 1O2 generation efficiency, negligible dark toxicity, and excellent tumor-targeting ability. Importantly, this complex totally inhibits the tumor
growth under 980 nm laser irradiation after the fourth injection.

4 | ANTIHYPOXIC TUMOR STRATEGY

Most Ir(III) complexes as PSs rely on the production $^1\text{O}_2$ to cause tumor cell apoptosis and necrosis through type II pathways, consequently their therapeutic effect is highly subject to the oxygen content in the tumor. Unfortunately, solid tumors is a hypoxic environment, which stems from the imbalance between the malformed cancer cells proliferation and deficient vascular oxygen supply. The hypoxic environment limits the antitumor effects of PSs, especially the need for continuous treatment. Therefore, there is an urgent need to develop smart of Ir(III) complex as PS to overcome the shortcomings of hypoxia and achieve more ideal tumor treatment.

In 2016, Huang et al. designed two Ir(III) complexes that specifically targeted mitochondria ($\text{Ir-P(ph)}_3$) and lysosomes ($\text{Ir-alkyl}$), respectively (Figure 10A). The obtained two complexes both exhibit similar $^1\text{O}_2$ quantum yield, long-lived phosphorescence and highly sensitive to oxygen quenching. With the oxygen concentration decreases, their luminous intensity increases and lifetimes extended (Figure 10B). Compared with targeted lysosomes-targeted complexes, cells treated with mitochondrial-targeted complexes can retain a slower respiration rate, which leads to higher intracellular oxygen levels under hypoxic conditions (Figure 10C), thereby increasing its hypoxic antitumor effect. MTT experiment shows that $\text{Ir-P(ph)}_3$ possess excellent PDT effect under hypoxic conditions, confirmed that mitochondria-targeted PDT agents have a positive effect in the treatment of hypoxic cancer.

Recently, Chao et al. reported for the first time an oxygen-independent Ir(III) complex as PS for two-photon PDT (Figure 10D). The introduced anthraquinone group can effectively trigger the Ir(III) complex to produce highly cytotoxic carbon radical, and can effectively quench its luminescence. Under hypoxic conditions, the emission of Ir(III) complex containing the anthraquinone group is turned-on after reduction by reductase (Figure 9E). More importantly, the reduced form of Ir(III) complex can effectively generates carbon radicals after irradiation (730 nm), causing the loss of mitochondrial membrane potential and cell apoptosis.

Very recently, Mao’s groups designed cyclometalated Ir(III)-metformin conjugates. This complex quickly penetrates into cancer cells and modulates the hypoxic microenvironment, resulting in about 10-fold higher cytotoxicity than conventional cisplatin. Moreover, this complex combined with metformin further endows the Ir(III) complexes with antimetastasis and anti-inflammatory activity. Therefore, the conjugation by metformin and Ir(III) complexes can greatly improve the antihypoxic tumor effect.

Except for tumor, the transition Ir(III) complexes can be used as the PSs for the other diseases such as Alzheimer’s disease and inflammatory diseases. In general, the transition Ir(III) complexes has the ability to alter peptides due to its hydrolytic cleavage and oxidation. Hence, a single Ir(III) complex was used to modulate the Aβ peptides in Alzheimer’s disease. This Ir(III) complex
greatly regulates the aggregation pathways of two main Aβ isoforms, A\(\beta\)\(_{40}\) and A\(\beta\)\(_{42}\), as well as the production of toxic A\(\beta\) peptides. Also, due to the excellent photophysical, photochemical, and electrochemical properties, Ir(III) complexes have attracted increasing interest in the development of responsive chemosensors in inflammatory diseases. The Ir(III) complexes has four advantages: (1) high photostability for real-time monitoring of targets without photobleaching; (2) large Stokes shift for minimizing the possibility of self-quenching; (3) long luminescence lifetime for lifetime imaging and time-gated luminescence imaging; (4) low cytotoxicity for good applications in biological samples. Taking the advantages of Ir(III) complexes, optical and electrochemical analyses of the response of Ir-Fe towards HOCl are investigated. The results demonstrated that Ir-Fe is an effective chemosensor for imaging of HOCl generation in liver injury in vivo and inflammatory diseases in mammalian bodies.

5 SUMMARY AND FUTURE PERSPECTIVES

Altogether, the transition Ir(III) complexes are developing fast due to high ISC ability, tunable optical properties, long excited state lifetimes, bringing encouraging performances. In this review, we summarized the recent progress of Ir(III) complexes in PDT. The NPs strategy can significantly improve its biocompatibility and cell internalization efficiency, thereby enhancing the effect of PDT. In addition, the Ir(III) complexes with long wavelength exhibits deeper tissue penetration. Constructing mitochondria-specific PS has a significant positive effect on enhancing the therapeutic effect of antihypoxic tumors. For future applications, we particularly highlight the following two areas which are at the forefront of exciting advances in Ir(III) metal complexes:

5.1 Photothermal therapy (PTT)

Currently, transition Ir(III) complexes have made remarkable achievements in the biological field. However, there are few reports on their application in photothermal therapy (PTT), which severely limits their use in phototherapy. More importantly, PTT is independent of the oxygen concentration, and is, thus, an excellent candidate to treat hypoxic tumors, which will effectively make up for the deficiency of PDT. Therefore, achieving photothermally active Ir(III) complex as PS is a very important goal to effectively overcome the oxygen dependence in PDT, and ultimately to improve the outcome of phototherapy. For
the further, constructing strong functional D-A groups into Ir(III) complexes is important to enhance the TICT effects and promote the formation of PTT. In addition, the introduction of a fluorescent chromophore with strong NIR absorption to extend the excitation light of Ir(III) complex will retain high-efficiency PTT effects.

5.2 Chemiluminescent Ir(III) complex

The phototherapyeffectIr(III)complextriggeredbylong-wavelength light is far from idea due to the poor energy conversion efficiency. Chemiluminescence, as a new light source without external light source, has recently gained increasing attention in recent years because of the minimize background interference, deep tissue penetration, and excellent sensitivity. The combined advantages of chemiluminescence and excellent ISC properties from Ir(III) complex, should lead to highly efficient deep PDT.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR BIOGRAPHIES

Liping Zhang received her’s PhD degree from the College of northeast normal university in 2020. She is currently a Postdoctoral fellow under the supervision of Prof. Dan Ding in the State Key Laboratory of Medicinal Chemical Biology in Nankai University. Her current research focuses on the design and synthesis of smart/functional nanomaterials, and the exploration of their biomedical applications.

Dan Ding received his PhD degree from the Department of Polymer Science and Engineering in Nanjing University in 2010. After a Postdoctoral training in the National University of Singapore, he joined Nankai University, where he is currently a Professor in State Key Laboratory of Medicinal Chemical Biology, Key Laboratory of Bioactive Materials, Ministry of Education, and College of Life Science. He also conducted his work in Hong Kong University of Science and Technology as a visiting scholar. His current research focuses on the design and synthesis of smart/functional molecular imaging probes and exploration of their biomedical applications.

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