Introduction

Liver cancer, which primarily includes hepatocellular carcinoma (HCC), cholangiocarcinoma, or mixed carcinoma, originates in the liver and intrahepatic bile duct. Liver cancer is the second leading cause of cancer death worldwide, following lung cancer, with a remarkably high mortality-to-incidence rate of up to 0.95, according to statistics (1). Currently, surgical resection is the most efficient and mainstay treatment for liver cancer. Other treatment modalities, such as radiofrequency ablation (RFA), trans-arterial chemoembolization, percutaneous ethanol injection, percutaneous microwave coagulation therapy, radiotherapy, and systemic chemotherapy, have been introduced for liver cancer treatment (2-4). Although considerable advancement in liver cancer treatment modalities has been attained, many patients with unresectable liver malignancies still suffer from a low survival rate. Therefore, it is meaningful to discover new therapeutic agents, cancer detection methods, and novel treatment modalities under these situations.

Nanoparticles (NPs) gain much popularity due to biological, therapeutic and medical applications in modern times (5). Iqbal et al. synthesized silver oxide NP via chemical aqueous method and found it could inhibit the cell viability of HepG2 cell line effectively (6). To improve the...
Second, PDT is a painless and simple method that can be used for outpatients. Third, PDT can be applied along with other palliative therapies, such as chemotherapy and radiotherapy (23). Although PDT has been proven to be an effective clinical therapy for cancers such as liver malignancies, its utility has been limited for several reasons: (I) the absorption of specific photosensitizers may be greater in normal liver tissues than in liver malignant tumors, thereby reducing the sensitivity and specificity of this therapy for liver tumor; (II) as liver has a large volume and pigmented tissues, the shallow penetration of light affect the phototoxicity of PDT (13); (III) because of the deep location of liver in the abdominal cavity, it is difficult for the transmission of laser fiber to the tumor. Rapid progress has been made by research groups in photosensitizer modification, light source improvements, and new drug delivery development to solve these problems and facilitate the application of PDT to liver tumors.

In this review, we will summarize the contemporary practices of PDT for liver malignancies.

PDT for HCC

HCC accounts for most liver cancer cases, posing a severe global public health problem (2). Surgical resection by removal of cancerous tissues offers the best long-term outcome in HCC patients, but curative therapeutic option is limited for patients with advanced or terminal HCC. Thus, a new therapeutic method is urgently needed.

PDT has been demonstrated as an effective method for treating HCC (24-28). Multiple mechanisms are involved in PDT-mediated tumor cell killing for HCC in vitro and vivo. To measure the mechanism of cell toxicity of PDT on liver cancer cell lines, Shi R and his colleagues (29) used sinoporphyrin sodium as a photosensitizer toward human HCC, which included bel7402 and HepG2 cell lines. The experiment indicated that PDT might be induced by injury to the mitochondria, which then initiated apoptotic responses, such as cytochrome c release into the cytoplasm and caspase protein activation. Tang et al. (30) demonstrated that with phophorbid a as a photosensitizer, PDT on human HCC can inhibit multi-drug resistance by down-regulating the expression of P-glycoproteins via c-Jun N-terminal kinase activation in vitro. They also noted that PDT treatment mediated by phophorbid a was more efficient in inhibiting the growth of Hep3B cells than that of the normal hepatic cell line WRL-68; PDT possibly targeted the mitochondria, which were more active in
cancer cells than in normal cells, to generate ROS near the mitochondrial membrane (31). As a result, the altered potential of the mitochondrial membrane led to the release of cytochrome c from the mitochondria to the cytoplasm and initiated apoptosis. Another programmed cell death pathway, autophagy, can exert cytoprotective function when apoptosis occurs during PDT (32). Recently, Domagala et al. reported that the inhibition of autophagy could sensitize Hela and MCF-7 cancer cell lines to photofrin-based PDT (33). However, whether the same mechanism exists in HCC cell lines remains unknown. Therefore, further studies are needed to clarify the role of autophagy in PDT for HCC. PDT was also found to be linked with immune responses. Zhang and his colleagues (34) established a disease model by injecting a H22 cell suspension into Kunming mice; they observed that an increase in the population of CD4⁺, CD8⁺, and CD19⁺ cells was linked to the tumor growth inhibition after administering the PDT-generated vaccine. They reported that PDT-generated vaccines could be used as an adjuvant therapy for cancer. PDT could also trigger inflammatory responses in addition to promoting apoptosis. It was reported that hypericin-mediated PDT can cause the death of HepG2 cells by regulating the expression of apoptosis-associated genes, such as caspases and cytochrome complex. Also, a remarkable increase of interleukin-6 was observed, whose expression level showed a close link with the apoptosis of tumor cells and caspase activity (27).

Positive surgical margin is an important reason for the recurrence of HCC after surgery (35). Intraoperative PDT is used as a concomitant and adjuvant therapy that can kill possible residual tumor cells in the surgical margin of liver to reduce the possibility of tumor recurrence. Muragaki et al. reported a phase II clinical study on intraoperative PDT with talaporfin sodium and semiconductor laser in patients with malignant parenchymal brain tumors (36). The 12-month overall survival and 6-month progression-free survival of the included 22 patients reached 95.5% and 91%, respectively. The authors concluded that intraoperative PDT may be a potentially effective and sufficiently safe option for adjuvant treatment of malignant brain tumors. However, no other similar studies have reported the intraoperative PDT of HCC. With the development of laparoscopic instruments and surgical techniques, PDT for HCC can be conducted during minimally invasive operations (Figure 1A), that is, laparoscopic-assisted PDT. The laser can be transmitted to the lesions through a 12 mm diameter trocar. Two main endoscope technologies exist for laparoscopic-assisted PDT (37): laser fiber separating from the endoscope and the combination of optical fiberscope and laser fiber. The latter integrates the two parts, and is the ideal instrument to develop in the future for laparoscopic-assisted PDT. However, one disadvantage of laparoscopic-assisted PDT is that the laser irradiation field is difficult to control, possibly injuring the surrounding normal tissues, and thereby causing serious complications.

**PDT for cholangiocarcinoma**

Cholangiocarcinoma, a rare malignancy originating from epithelial cells of the biliary tree, remains the second most common primary liver cancer, following HCC (38). Cholangiocarcinoma is relatively difficult to diagnose given its deep location and the lack of definitive diagnostic criteria (39). Most patients present inoperable cholangiocarcinoma at the time of diagnosis, and their survival is measured in months (median survival: around 5 months) (40). Long survival time is only associated with a R0 margin in resection, whereas the five-year survival rate reaches 30–40% after curative resection (41,42). Therefore, palliative treatment is needed for most cholangiocarcinoma patients.

PDT is an effective palliative method for advanced bile duct carcinoma (43-47). The first randomized controlled trial (RCT) to study the effect of PDT on nonresectable cholangiocarcinoma was reported in 2003 (48). The research compared cholangiocarcinoma patients treated by PDT and stent with those who received a stent only. The results suggested that the combination of PDT and stent offered better treatment outcomes for nonresectable cholangiocarcinoma than stent only. Another RCT was conducted for 32 patients with nonresectable bile duct cancer in 2005 (49). In the PDT group, photosan-3 (R) at a dosage of 2 mg/kg body weight was administrated, whereas the control group was treated with endoprosthesis. The median survival time of the control group was 7 months, whereas that of the PDT group was significantly longer at 21 months (P<0.05). These RCT studies provide high-quality evidence for the use of PDT in patients with cholangiocarcinoma.

Chemotherapy is an important treatment modality for cholangiocarcinoma (50). The combination of chemotherapy with PDT for cholangiocarcinoma can theoretically obtain better treatment effect than PDT alone. Nonaka et al. (51) reported that the combination therapy of PDT with gemcitabine and oxaliplatin showed synergic
effects on necrosis, apoptosis, and cytostatic alterations in advanced bile duct carcinoma. A randomized phase II trial reported by Park et al. (52) revealed that PDT plus S-1 was associated with better overall survival and progression-free survival than PDT alone in patients with unresectable hilar cholangiocarcinoma. Another retrospective study enrolled 74 patients with hilar cholangiocarcinoma (53). Among these patients, 16 were treated with PDT and gemcitabine with or without cisplatin, whereas 58 were treated with PDT only. The results showed that PDT with chemotherapy achieved a longer survival time than PDT alone. Wentrup et al. (54) reviewed 68 patients with hilar nonresectable cholangiocarcinoma treated with either PDT plus chemotherapy or PDT monotherapy. They observed that the PDT plus chemotherapy group achieved a significantly higher one-year survival rate than the PDT monotherapy group (P=0.001). In general, the tumoricidal effect of PDT is often limited to the inner 4–6 mm of the tumor wall (55). However, most cholangiocarcinomas are locally advanced when diagnosed, and the lesions may be thick. Meanwhile, micrometastasis or distant metastasis may exist for advanced cholangiocarcinoma. PDT, which primarily plays a role in local regions, may feature limited therapeutic effects for patients with such conditions. Therefore, systemic chemotherapy may be used to obtain survival benefit for the patients with cholangiocarcinoma receiving PDT.

Biliary drainage can be beneficial as a palliative treatment in patients with unresectable cholangiocarcinoma, with longer survival time and less cost than surgical treatment (56). Leggett et al. (57) used meta-analysis to compare the overall outcome and effectiveness of biliary stenting combined with PDT with that of biliary stenting therapy alone in patients with cholangiocarcinoma. They revealed that the palliative treatment of cholangiocarcinoma with PDT is associated with survival benefits, that is, the improved biliary drainage and quality of patients’ life. The following meta-analysis conducted in 2015 obtained similar
results (58). Another meta-analysis that included 10 studies discovered survival periods of 413.04 and 183.41 days in PDT plus biliary stenting group and biliary stenting only group, respectively (59). The author concluded that PDT plus stenting is superior to stenting alone and recommended PDT as an adjunct to biliary stenting in patients with unresectable cholangiocarcinoma.

When performing PDT on cholangiocarcinoma, endoscopic retrograde cholangiopancreatography (ERCP) and percutaneous transhepatic cholangioscopy (PTCS) are two common delivery methods for laser radiation (60) (Figure 1B). Both approaches are minimally invasive, and they feature a low incidence of adverse events. A recent study reported that the overall survival after PTCS- or ERCP-directed PDT showed no statistically difference (11.6 versus 9.5 months, P=0.96) in patients with advanced hilar cholangiocarcinoma (61). ERCP-directed PDT presents several advantages (61): (I) multiple segments can be treated in one time; (II) compared with PTCS-directed PDT, ERCP-directed PDT requires no waiting time for sinus tract maturation to allow the passage of cholangioscopy. However, limitations also exist for ERCP-directed PDT: (I) the appropriate location for placement of the fiber and probe used in PDT can be difficult to determine because extrahepatic bile duct carcinoma spreads longitudinally (62); (II) ERCP-directed PDT cannot accurately evaluate the response to treatment because it is performed under fluoroscopy guidance; (III) the patients may hesitate in accepting the peroral transpapillary approach; (IV) ERCP-directed PDT can more likely cause serious complications, such as acute pancreatitis and hematobilia, than PTCS-directed PDT. Meanwhile, given that PTCS-directed PDT is performed using direct endoscopic visual control, it can provide effective, visual, and homogenous irradiation of the targeted lesion, and it can be monitored repeatedly without periodic peroral endoscopy. Nevertheless, PTCS-directed PDT also presents drawbacks: (I) the PTBD tube should be left in place for a long time until the final PDT session to maintain the patency of the PTBD tract, thereby causing inconvenience to patients; (II) the PTBD procedure may cause peritoneal dissemination of cancer cells in the bile. In addition to ERCP and PTCS, laser fibers can also be introduced through the T-tube sinus for patients receiving laparotomy (Figure 1C), which can be convenient for patients to receive repeated PDT. In summary, PDT offers an important option for patients with cholangiocarcinoma, especially the unresectable type.

PDT for hepatoblastoma

Hepatoblastoma is the most common primary liver cancer in infants and children, accounting for around 1.5% of all incidence of pediatric malignancies (24). A low long-term survival rate is observed in patients with unresectable advanced hepatoblastoma (63). Hypericin, a photosensitizer derived from the natural products of flowering plants belonging to Hypericum (64), exhibits a stable fluorescence, desirable tissue penetration, preferential tumor retention, and greater cytotoxic effects in tumor cells than in normal tissues (65). This photosensitizer shows potential application in PDT for hepatoblastoma because it causes no severe side effects (66). Seitz et al. (24) demonstrated that the enhancement of concentration of hypericin in two hepatoblastoma cell lines, namely, HuH6 and HepT1, resulted in decreased tumor cell viability. Future studies are needed to establish the use of hypericin-PDT for hepatoblastoma in vivo. Bergmann et al. (67) used 5-aminolevulinic acid (5-ALA) as a photosensitizer and demonstrated a marked and specific fluorescence in human hepatoblastoma (HuH6) in vitro. They also observed that human hepatoblastoma was susceptible to PDT in a nude rat model. Curcumin is a yellow-orange dye derived from the rhizome of Curcuma longa. This compound is one of the most extensively studied phytochemicals. Curcumin could facilitate the absorption of radiation between 350–500 nm and cause oxygen-dependent phototoxicity (68,69). Curcumin-mediated PDT could enhance the anti-tumor properties of curcumin in hepatoblastoma cell lines (HuH6 and HepT1) by inducing the loss of viability via ROS production (70).

In general, surgical resection offers the mainstay of therapy for hepatoblastoma. For unresectable cases, chemotherapy is an important palliative treatment that significantly improves the overall survival period (71). Thus, PDT offers an alternatively effective method for treating hepatoblastoma, although further in vivo studies are needed.

PDT for metastatic liver cancer

Metastatic liver cancer, also known as secondary liver cancer, mostly develops from colorectal cancer. The median survival time of patients with colorectal liver metastases is 6–9 months if untreated (72), whereas the rate of operable colorectal liver metastases only reaches 10% in all patients (73). To date, surgical removal of metastatic tumor
is the only curative treatment method. Other treatments, such as RFA, chemoradiotherapy, and PDT, are alternative modalities. PDT may be specifically suitable for patients with unresectable liver metastases or with liver metastases in the vicinity of large vessels.

Some findings indicate that PDT for metastatic liver cancer can achieve satisfactory effects (13,74,75). Interstitial PDT (IPDT) is a good choice for deeply seated tumors or tumors thicker than 10 mm (76) (Figure 1D). IPDT involves the insertion of laser fibers directly into the tumors. The laser fibers can be inserted via needles, or placed in catheters (76). Light can be delivered through the end of the fibers, or through a fiber with a cylindrical diffuser end. To evaluate the IPDT for the treatment of solid liver tumors, researchers used LS 11 (talaporfin sodium with an applied dose of 40 mg/m²) as a photosensitizer to treat four metastatic liver cancers of four patients (three colorectal carcinoma and one melanoma). The LS 11 was activated via CT-guided percutaneously inserted intratumoral Lumaflex Light Sources. The mean diameter of tumor necrosis was around 14 mm (range, 13 to 17 mm), and no cutaneous phototoxicity was observed (77). Bacteriochlorin a (BCA) is a nontoxic photosensitizer derived from bacteriochlorophyll a, and early researchers have discovered its preferential retention in certain types of tumors. Subsequently, a study reported that the BCA concentrations in normal liver and tumor of a Wag/Rij rat model implanted with colon carcinoma CC531 cells failed to show tissue preferences after the intravenous injection of BCA (10 mg/kg) and interstitial irradiation of 760 nm laser light (78). The author also revealed that the concentration of BCA showed no significant fluctuation but declined rapidly in the first 4 h. This finding implied that optimal results with IPDT could be obtained by illumination within a short interval after the administration of BCA (78). Another experiment used Photofrin for IPDT in a rat liver metastasis model, with the colon carcinoma CC531 cells implanted into the Wag/Rij rat model. The results confirmed that IPDT using Photofrin as a photosensitizer could cause the major destruction of tumor tissues with minimal liver damage (79). The main advantage of IPDT is that the light delivery from multiple fibers enables the treatment of large and deep tumors, which cannot be illuminated with external beam PDT (76).

Altogether, these findings provide the novel aspects of PDT modality in the treatment of metastatic liver cancer.

**Photosensitizers for anti-liver malignant tumors**

The modifications of PDT are focused on the type of photosensitizer, the intensity and wavelength of light controlling the tumor tissue penetration, the light delivery devices, and the interval between the administration of photosensitizer and irradiation (80). The most currently used photosensitizers exhibit absorption peaks at the 600–750 nm range in experimental and clinical PDT for liver malignancies (81). Given the large volume of liver, the PDT triggered by long-wavelength light is meaningful for the treatment of liver cancer. Several methods that can improve light delivery and intensity have been proposed for the treatment of deep cancers (82). The first method involves the development of photosensitizers activated by near-infrared (NIR) light, which presents a stronger tissue penetration than ultraviolet or visible light. The second method uses upconversion nanoparticles (UCNPs), which can be excited at NIR and then emit light in the ultraviolet-visible range for PDT activation (83). Liang et al. (84) combined a new photosensitizer of KillerRed with UCNPs to obtain photosensitizing bio-nanohybrids, the KillerRed-UCNPs, which exhibited excellent colloidal stability in biological buffers and low cytotoxicity in the dark. They demonstrated that the KillerRed-UCNPs exhibited superiority over unbound KillerRed in vitro PDT model of MDA-MB-231 cells buried under ~1-cm pork tissue. The third method is the use of X-ray-induced scintillation or afterglow NPs for PDT activation (85).

Thus far, various photosensitizers such as hematoporphyrin derivative (26), pheophorbide a (86), ALA (87), sinoporphyrin sodium (29), and indocyanine green (88) have been evaluated and validated in clinical practice for the treatment of liver malignancies. Normally, an optimized photosensitizer is highly soluble and shows low toxicity before illumination and can produce maximum phototoxic effect in a restricted area around the tumor cells but not in the adjacent normal tissues (89).

NP-mediated targeted drug delivery system is a novel PDT approach that increases the tumor specificity of photosensitizer for liver malignancies. Tumor angiogenesis is a process in which new blood vessels in tumor tissues form from the existing ones. The tumor vasculature substantially differs from normal tissue blood vessels. The defective vascular architectures for tumors include discontinuous endothelial lining, lack of smooth muscle
cells, pericyte deficiency, and aberrant basement membrane formation, which lead to an enhanced vascular permeability (90,91). Nanocarriers (size range, 20–200 nm) can extravasate and accumulate inside the interstitial space, thereby resulting in their retention in tumors (92). This passive phenomenon is called the “enhanced permeability and retention (EPR) effect”. The EPR effect is now one of the most important approaches for passive targeting of PDT on tumors. A simple but effective NP system based on phospholipid, Pluronic F68 (PF68), and pullulan was designed and reported recently (93). A heptamethine dye IR780 and chemotherapeutic drug paclitaxel were loaded separately into NP system to form PDFI and PDFP NPs. In HCC cell line MHCC-97H, the combined treatment of PDFI and PDFP NPs exhibited significant synergistic effects on inhibiting cell proliferation and inducing cell apoptosis and cell cycle arrest at the G2/M phase. This study indicated that the new NP system could combine PDT with chemotherapy to treat HCC, offering a promising direction for the PDT of HCC. Iqbal et al. synthesized photosensitizers of zine-doped cobalt ferrite (Zn$_{0.5}$Co$_{0.5}$Fe$_2$O$_4$) NPs and Co$_3$O$_4$ nanocrystalline materials and found they could inhibit the cell viability of HepG2 cells effectively when exposed to appropriate wavelength light (94,95). Recently, a new photosensitizer called copper-cysteamine NPs (Cu-Cy NPs) was reported (96). Cu-Cy NPs-mediated PDT could notably inhibit HepG2 cells. The Cu-Cy NPs can enter into the exosomes secreted by tumor cells, and exosomes could be used to deliver Cu-Cy NPs to target tumor cells.

Several proteins and molecules can be overexpressed on the surface of liver tumor cells compared with normal cells. The ligands of these proteins and molecules, including (poly)saccharides, vitamins, antibodies, peptides or other small molecules, are utilized to decorate drug delivery systems (92,97). NPs or photosensitizers may exhibit tumor-targeting capability by surface modification of ligands for the receptors in liver tumor tissues (98) in a process called active targeting. Active targeting can further improve the targeting property of photosensitizers toward the liver tumor tissues (99). Table 1 provides active targeting studies for the PDT of liver malignancies.

### Table 1 Summary of active targeting for photodynamic therapy on liver malignancies

<table>
<thead>
<tr>
<th>Time</th>
<th>Ligands</th>
<th>Matching receptors</th>
<th>Photosensitizers</th>
<th>Delivery vehicles</th>
<th>Tumor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000 (100)</td>
<td>Monoclonal antibody 17.1A</td>
<td>Ep-CAM</td>
<td>Chlorine6</td>
<td>Anionic 17.1A conjugate</td>
<td>HT29</td>
</tr>
<tr>
<td>2010 (101)</td>
<td>Anti-HSA monoclonal antibody</td>
<td>Hepatocyte specific antigen (HAS)</td>
<td>Hypericin</td>
<td>Anti-HSA-Hyp</td>
<td>HepG2</td>
</tr>
<tr>
<td>2011 (102)</td>
<td>Low-density lipoprotein</td>
<td>Low-density lipoprotein receptor</td>
<td>Bacteriochlorin e6</td>
<td>r-Bchl-BOA-LDL</td>
<td>HepG2</td>
</tr>
<tr>
<td>2014 (103)</td>
<td>Hematoporphyrin</td>
<td>Low density lipoprotein receptor</td>
<td>Hematoporphyrin</td>
<td>HP-NPs</td>
<td>HepG2</td>
</tr>
<tr>
<td>2015 (104)</td>
<td>Anti-Glypican-3 antibody</td>
<td>Glypican-3</td>
<td>IRDye700DX</td>
<td>IR700-YP7</td>
<td>A431/G1</td>
</tr>
<tr>
<td>2016 (105)</td>
<td>Anti-EGFR antibody</td>
<td>EGFR</td>
<td>Chlorin e6</td>
<td>Ce6-IgG-QDs</td>
<td>HepG2</td>
</tr>
<tr>
<td>2017 (106)</td>
<td>Aptamer TLS11a</td>
<td>A membrane protein</td>
<td>Chlorin e6</td>
<td>Ce6-fDNA$_{ox}$</td>
<td>HepG2</td>
</tr>
<tr>
<td>2017 (93)</td>
<td>Pullulan</td>
<td>Asialoglycoprotein receptor</td>
<td>IR780</td>
<td>PDFI NPs</td>
<td>MHCC-97H</td>
</tr>
<tr>
<td>2019 (107)</td>
<td>Aptamer TLS11a</td>
<td>A membrane protein</td>
<td>Black phosphorus quantum dots</td>
<td>Apt-BMSF@Pt</td>
<td>HepG2</td>
</tr>
</tbody>
</table>

### Conclusion

PDT has been applied for patients with liver cancer and has shown clinically beneficial effects. However, the liver is a special metabolic organ that possesses a remarkable capacity for regeneration. In addition, the liver features a large volume, and it is deeply located in the abdominal cavity. Difficulty arises in the development of appropriate photosensitizers, which are triggered by long-wavelength light and highly selective between normal tissues and cancerous cells in the liver. Additionally, PDT studies on humans remain inadequate, and better delivery methods for laser radiation are needed. Future research should develop new photosensitizers, such as NPs or active targeting of photosensitizers specific to liver malignancies.
Meanwhile, the development of endoscopy-assisted PDT and IPDT is meaningful to improve the efficacy of PDT on liver malignancies. Patients receiving PDT along with other palliative methods, such as RFA, chemotherapy, or biliary stenting, can obtain better results than PDT alone. In summary, PDT can be used as a palliative treatment modality to improve the quality and duration of life of patients with liver malignancies. On this basis, PDT can play a positive role in the treatment of liver malignancies.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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