*Review Article*

# Perspectives on the Role of Photodynamic Therapy in the Treatment of Pancreatic Cancer

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Photodynamic therapy (PDT) is a noninvasive procedure involving a photosensitizing agent that is activated by light to produce reactive oxygen species (ROS) that selectively destroy tumor cells. In recent years, PDT has been used in the treatment of pancreatic cancer (PC). The antitumor effects of PDT include three main mechanisms: direct tumor cell death (necrosis, apoptosis, and autophagy), vascular destruction, and immune system activation. The present paper systematically summarizes the effects of PDT in the treatment of PC from the experimental studies to the clinical studies and discusses the mechanisms of PDT-induced PC destruction.

## 1. Introduction

Pancreatic cancer (PC) is one of the most lethal malignant diseases and has a dismal prognosis. It is estimated that over 37,000 patients were newly diagnosed with PC, and 34,000 patients died of this disease in the USA in 2010 [1]. PC has the lowest 5-year survival rate of any gastrointestinal tumor, and the median survival rate is no more than 6 months [1, 2]. Surgery remains the only way to cure this disease, but less than 20% of patients are considered for surgical resection at the time of initial diagnosis [3]. Moreover, even seemingly resectable PC often fails to cure the disease due to the microscopic systemic spread of the cancer that occurred before the operation [4]. Current treatments for inoperable patients are still limited to chemotherapy, radiation, or both (chemoradiotherapy) [5]. A new comprehensive and constructive therapy is urgently needed.

Photodynamic therapy (PDT) is a treatment that uses nontoxic drugs or dyes (photosensitizers) that are pharmacologically active only after exposure to light in the presence of oxygen [6, 7]. Due to its fundamental selectivity and specificity [8], PDT has been considered to be a possible treatment for neoplasms, including cancers of the skin [9], head and neck [10], nasopharynx [11], esophagus, lung [12], pancreas, biliary duct [13], and bladder [14]. The four main kinds of photosensitizers are porphyrin derivatives, chlorines, phthalocyanines, and porphycenes [7, 15] (Figure 1). The photosensitizer excited triplet state undergoes two broad kinds of reactions (Type I and Type II). In a Type I reaction, the triplet photosensitizer can transfer an electron to a neighboring substrate to form free radicals and radical ions, which further interact with molecular oxygen and produce reactive oxygen species (ROS). In a Type II reaction, the triplet photosensitizer can transfer its energy directly to molecular oxygen and form excited-state singlet oxygen [16, 17] (Figure 2). These two reactions constitute the core mechanism of PDT-mediated destruction of tumor tissue. In recent years, more and more research has focused on the development of PDT for the treatment of PC [18].

## 2. Experimental Studies

*2.1. Mechanisms of Pancreatic Cancer Cell Death in PDT.* Cells can undergo three distinct kinds of cell death induced by PDT: necrosis, apoptosis, and autophagy [7, 19]. Necrosis is morphologically characterized by increased cellular volume, swelling of organelles, plasma membrane rupture and the subsequent loss of intracellular contents [20]. It is

Porphyrin                                    Chlorin                                    Porphycene



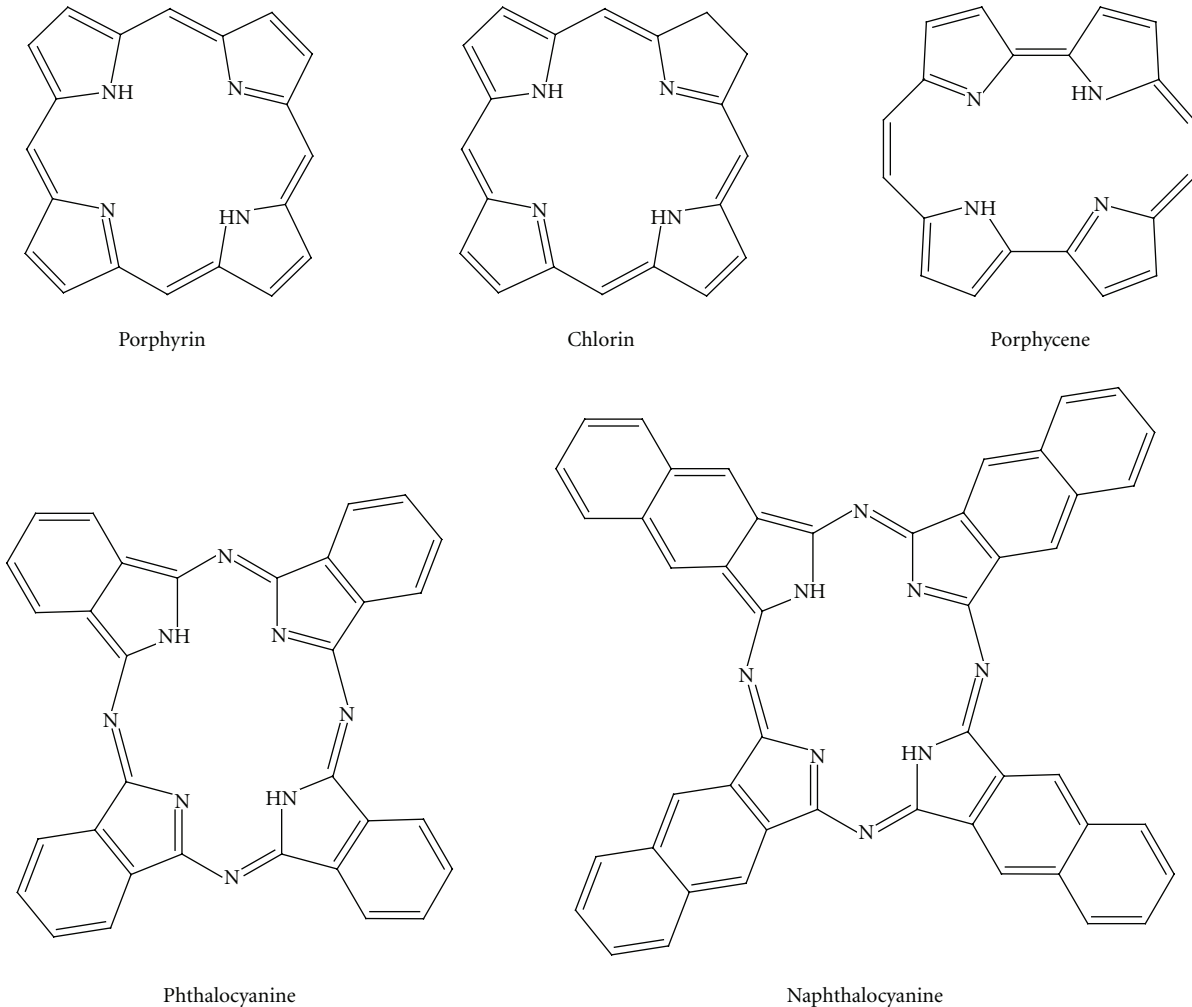Phthalocyanine                                                    Naphthalocyanine

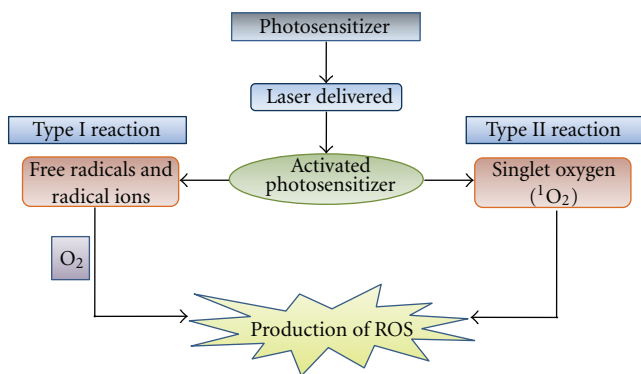FIGURE 1: Chemical structures of major PDT photosensitizers.



FIGURE 2: Mechanisms leading to the formation of ROS. There are two types of reactions during PDT. In Type I reaction, the triplet photosensitizer reacts with the neighboring substrate and forms free radicals as well as radical ions, which further interact with molecular oxygen and produce ROS. In Type II reaction, the triplet photosensitizer transfers its energy directly to molecular oxygen and form excited-state singlet oxygen.

generally believed that higher light dosage is always accompanied by cellular necrosis [21]. Using different orthotopic pancreas cancer xenograft models (AsPC-1 and Panc-1), Samkoe et al. [21] demonstrated that both Panc-1 tumors and AsPC-1 tumors became necrotic following treatment with verteporfin PDT and hematoxylin/eosin-stained tumor slices displayed increasing necrotic/edemic core with the increase of light dose. Besides, faster growing tumors (AsPC-1 cell line) were relatively easier to treat.

Another *in vivo* experiment reported by Xie et al. [22] showed that PDT led to necrosis in cancer lesions and significantly reduced tumor volume. They observed that partial tumor necrotic tissue was exfoliated and a necrotic edge of volcano-like uplift was formed 1 week after PDT treatment. In a randomized, controlled study of implanted pancreatic cancers in Syrian golden hamsters treated with 5-aminolaevulinic acid, PDT-induced tumor necrosis of up to 8 mm in depth was achieved, and the survival time of

the treated animals was significantly longer than in the untreated control group [23].

Mlkvy et al. [24] conducted experiments to assess the effects of meta-tetrahydroxyphenylchlorin (mTHPC) in a hamster pancreatic cancer model. In their experiment, 0.1 or 0.3 mg/kg mTHPC was given to the animals, and the tumor was treated 2 or 4 days later via laparotomy with red light (50 J at 650 nm, continuous or fractionated) delivered through a single fiber touching the tumor surface. The results showed that the zones of tumor necrosis, often haemorrhagic in the center, sharply demarcated from adjacent viable tumor with an inflammatory infiltrate in the surrounding area. This may be attributed to the vascular supply pattern of the tumor or the protective role of surrounding connective tissue strips dividing tumor into lobules [23, 24]. The results also elucidated that the maximum zone of tumor necrosis was 8.7 mm in diameter with continuous irradiation, which increased to 12.4 mm with fractionated treatment.

In addition, PDT has also been proven to damage DNA. Hajri et al. [25] demonstrated that PDT inhibited tumor cell growth in cell culture by affecting DNA integrity. The DNA-damaging effects of PDT are related not only to variables in PDT but also to cellular repair and survival mechanisms [26]. Ferreira et al. [27] designed synthetic oligonucleotides (aptamers) that were selected to bind to unique short O-glycan-peptide signatures on the surface of pancreatic cancer cells and observed a remarkable enhancement (>500-fold increase) in toxicity of PDT in the presence of these phototoxic aptamers.

Apoptosis, another type of cell death, requires ATP and is characterized by cytoplasmic shrinkage, reduction of cellular volume, condensation of the chromatin, and fragmentation of the nucleus [28]. Several pathways have been proven to play a role in cellular death. For example, the classic anti-apoptotic proteins in the Bcl-2 family can be downregulated after PDT. It is known that Bcl-2 is a molecular target of PDT using mitochondrion-targeting photosensitizers and can determine the sensitivity of cancer cells to apoptosis and the overall cancer response to PDT [29]. Lutetium-texaphyrin-mediated PDT can not only downregulate the expression of Bcl-2 and upregulate the expression of Bax in bovine retinal capillary endothelial cells, but also influence Bcl-xL and Bak proteins in human retinal pigment epithelial cells [30]. Using phthalocyanine photosensitizer Pc4-mediated PDT, He et al. [31] found that parental cells displayed a high incidence of apoptosis after PDT, whereas Bcl-2-transfected cells exhibited a much lower incidence of apoptosis as assessed by DNA fragmentation. Another apoptosis-related protein, cytochrome c, was released from mitochondria upon treatment with PDT [32, 33]. It is reported that the release of cytochrome c from mitochondria is controlled by proteins of the Bcl-2 family. Liu et al. [34] observed that cytochrome c was released from the mitochondria into the cytoplasm during PDT, and the mitochondria membrane potential ($\Delta\Psi m$) showed a loss of nearly 30% in human pancreatic cancer cells. After releasing into the cytosol, cytochrome c is able to initiate apoptotic signal events, activating caspase-9 and then caspase-6 and caspase-7, respectively [35]. A second pathway involved in cell death stems from PDT-activated

caspases and the subsequent cleavage of the DNA repair protein poly (ADP-ribose) polymerase [36]. Yet another pathway involves the Fas ligand (FasL), which belongs to the tumor necrosis factor (TNF) family. When FasL binds to its receptor, apoptosis is induced. Fas/FasL system could either signal the apoptosis directly through the activation of the caspase system or through mitochondria [37]. PDT has been proven to enhance FasL expression, leading to an increase in FasL signaling-dependent cell death in cancer cells. A recent study has shown that PDT induced apoptosis of nasopharyngeal, colon, and bladder cells is mediated not only by activation of Fas with the involvement of the FasL system, but also by the activation of a distinct caspase cascade [38]. The activation of the caspase cascade, caspase-8 and caspase-3, follows direct activation of Fas/FasL in PDT-induced apoptosis [38]. Calcium plays an important role in photodynamic drug action. PDT-induced increases in the levels of intracellular calcium may be associated with cell apoptosis [7]. Calcium chelators were shown to inhibit the PDT-induced release of cytochrome c, caspase-3 activation and apoptosis in Chinese hamster V79 cells, indicating that calcium indeed plays a role in PDT-induced apoptosis [39]. In a recent study, Chiou et al. [40] showed that verteporfin PDT could rapidly provoke hyperoxidative stress and caspase activity in HepG2 cells. In addition, the membrane integrity was decreased and permeability increased, resulting in a sudden influx of cytosolic calcium into the mitochondria. All these factors were treated as the arbitrator to initiate the lethal apoptotic process after verteporfin PDT. The increase in the intracellular calcium concentration upon photosensitization may occur via the influx of calcium through ion channels, the release of calcium sequestered in internal stores and/or the activation of ion exchange mechanisms [41].

Cellular adhesion is also associated with cellular apoptosis. Galaz et al. [42] demonstrated that the loss of E-cadherin-mediated cell adhesion after early photodamage triggers an apoptotic response. They also observed that the alteration in E-cadherin preceded the release of cytochrome c from the mitochondria to the cytosol as well as the activation of caspase 3. Blocking E-cadherin function with a specific antibody induced apoptosis. PDT can also downregulate the expression of vascular cell adhesion molecule-1 and intra-cellular cell adhesion molecule-1 [43]. A notable feature of PDT is to change the attachment between cancer cells and stroma or cancer cells themselves, which can be attributed to the damage of adhesion molecules [44].

In recent years, more and more researchers have expressed concern about the p53-mediated cytotoxicity of the PDT of cancer [45]. A direct evidence supporting the idea that p53 is involved in PDT response came from the work by Mitsunaga et al. [46]. In their research, they showed that the activition of caspase-3 and caspase-9 increased in wild-type human colon cancer cells. In contrast, it was significantly inhibited in Bax-null or p53-null cells which indicated that the caspase-dependent apoptosis induced by PDT was Bax- and p53-dependent. Lim et al. [47] evaluated the ability of PDT combined with a tumor suppressor factor, recombinant adenovirus p53 (AdCMVp53), to induce apoptosis as well as cell growth inhibition. They noticed that cotreatment with
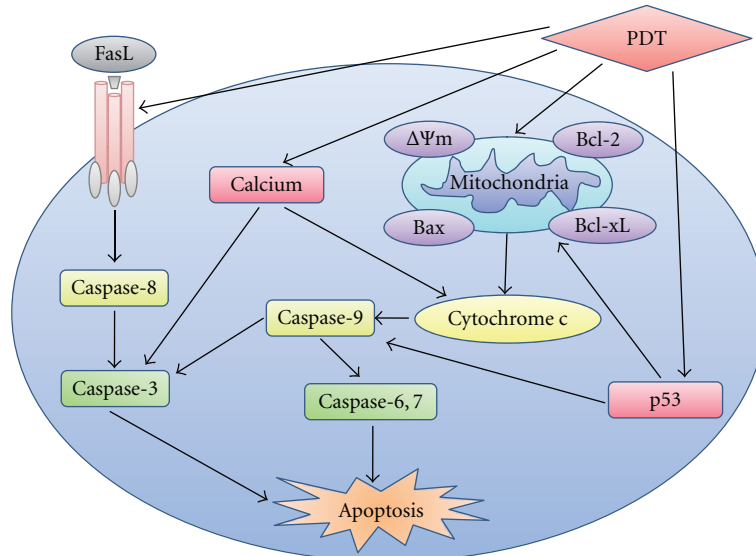
FIGURE 3: Schematic illustration represents the possible effect of PDT on the apoptosis pathways. PDT-associated apoptotic progress is a complicated occurrence which activates the mitochondrial pathway, promotes cytochrome c releases and caspase-3, -6, -7 activation. Moreover, PDT also influences cancer cellular apoptosis via the elevation levels of p53 and intracellular calcium.
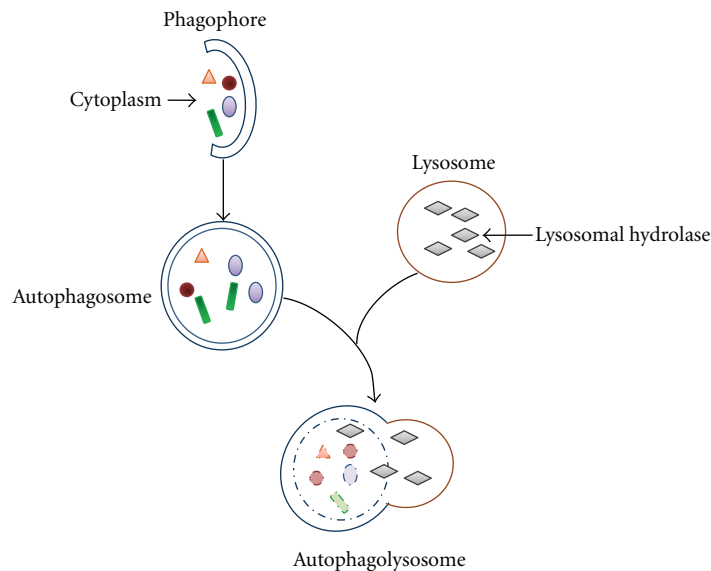


FIGURE 4: Schematic model of autophagy. First of all, a double membrane structure named autophagosome surrounds the target region and creates a vesicle which separates its contents from the rest of the cytoplasm. Secondly, the vesicle is transported and fused to the lysosome, forming autophagolysosome. Lastly, the contents are degraded by lysosomal hydrolases.

PDT and AdCMVp53 resulted in a more potent antitumor effect. Cotreatment led to elevated levels of p53, possibly causing the induction of p53-dependent apoptosis. It is generally believed that the key factors that determine the type of PDT-induced cell death are cell genotype, light dose, and the subcellular localization of the photosensitizers [7, 48] (Figure 3).

Autophagy is a process in which the abnormal cytoplasm is sequestered into double-membrane vesicles and fused by lysosomes, with the contents of the autophagosomes being digested and recycled [49, 50] (Figure 4). Due to the morphological and biochemical features of autophagic cell death, it is distinct from both apoptosis and necrosis [51]. Because autophagy develops in a sequential fashion, it is classified as a second type of programmed cell death. Both autophagy and apoptosis occur following PDT [19]. It is shown that autophagy is independent of photosensitizer target, because it is observed with photosensitizers localize in endoplasmic reticulum, mitochondria, lysosomes, and endosomes [52]. PDT can not only affect autophagy by damaging organelles

(lysosomes and endosomes) but also influence proteins that are involved in this mechanism [53, 54]. Using shRNA technology, Kessel and Arroyo [55] created a Bax knockdown line. A marked decrease in apoptosis was observed after photodamage or pharmacologic inactivation of Bcl-2 function in this cell line, but the PDT efficacy was not affected because the suppression of apoptosis leaded to enhanced autophagy (a highly vacuolated morphology). Autophagy appears to play a prosurvival role in apoptosis-competent cells and a prodeath role in apoptosis-incompetent cells [52].

### 2.2. Mechanisms of Pancreatic Tumor Destruction

*2.2.1. Vascular Destruction.* Pathological angiogenesis is a hallmark of tumor cells, and their viability depends on an adequate blood supply [56]. PDT-induced vascular damage is an important mechanism of tumor destruction. PDT-mediated vascular effects range from transient vascular spasm, vascular stasis, and the formation of thrombus to permanent vessel occlusion [57]. Vascular destruction may contribute to a reduction in tissue oxygenation and further promote tumor destruction [58]. Li and Luo [59] showed that the antitumor effects of PDT were achieved mainly by the destruction of tumor blood vessels and the formation of thrombosis at short drug-light intervals; in contrast, the tumor cells were killed directly by PDT-mediated cytotoxicity at long drug-light intervals.

Although the oxygen-consuming reaction of PDT mediates the destruction of tumor vessels, the hypoxic condition within tumors can cause the release of angiogenic growth factors and cytokines that could possibly decrease the efficacy of PDT by promoting tumor regrowth [60]. Zhou et al. [61] demonstrated that the expression of hypoxia-inducible factor (HIF)-1alpha, vascular endothelial growth factor (VEGF), and cyclooxygenase 2 (COX-2) were increased in PDT-treated tumors, indicating that PDT-induced damage to tumor microvasculature and the resultant hypoxia upregulated the expression of certain proangiogenic factors. Combining antiangiogenesis inhibitors along with PDT led to greater efficacy in cancer treatment [62]. In addition, through a concept called "arterial flow focalization," which allows for controlled temporary vascular occlusion of the collateral arterial branches upstream of the tumor, it is possible to redirect blood flow through the principal artery of the downstream tumor, thereby increasing tumor arterial flow and hence oxygen supply, thus further greatly improving the efficacy of PDT [63].

*2.2.2. Immune System Activation.* PDT-induced necrosis of tumor cells with the subsequent induction of an inflammatory response leads to antitumor immune responses [64, 65]. It has been reported that PDT alters the tumor microenvironment by stimulating the release or expression of various proinflammatory and acute phase response mediators [66–68]. In response to many kinds of stress, cells produce heat shock proteins (HSPs), and it is believed that PDT can induce the cell surface expression and release of HSPs, which in turn stimulates the inflammatory and immune

responses [69]. The body recognizes PDT-inflicted tumor tissue injury, and this further provokes a strong host response with neutrophilia as one of its manifestations [67]. In a rhabdomyosarcoma-bearing rat model, de Vree et al. [70] showed that PDT resulted in an increase in circulating neutrophils and the slowing of tumor growth. Depletion of neutrophils decreased the PDT-mediated effect on tumor growth. Antitumor immunity depends upon the presence of activated antigen presenting cells (APCs). PDT can increase the activity of APC and stimulate T-cell proliferation and T-cell secretion of interferon gamma [71]. The complement system is a biochemical cascade that consists of more than 30 serum and cell surface proteins [72]. The activated complement system was identified as an important element of the host response elicited by PDT [73, 74]. The complement system not only acts as a direct mediator of inflammation, but it also stimulates at least a dozen secondary inflammatory molecules, such as cytokines, interleukin-1beta (IL-1beta), TNF-alpha, IL-6, IL-10, granulocyte colony-stimulating factor, thromboxane, prostaglandins, leukotrienes, histamine, and coagulation factors [75]. Mroz et al. [76] recently showed that an effective vascular PDT regimen that can reliably promote local tumor destruction can also lead to antigen specific antitumor immunity. This tumor-destructive effect was mediated by tumor antigen-specific cytotoxic T cells. Moreover, PDT combined with low-dose cyclophosphamide can produce tumor-specific cytotoxic T cells and potent memory immunity, which in turn cause a dramatic improvement in survival and remission rates in a highly metastatic mouse tumor model [77] (Figure 5).

## 3. Clinical Studies

The first clinical trial of PDT in the treatment of locally advanced PC took place in 2002 [78]. In this phase I study, 16 inoperable patients with cancer in the head of the pancreas were treated with mTHPC (0.15 mg/kg). After 3 days, light was delivered to the cancer percutaneously using fibers positioned under ultrasound or computerized tomographic guidance. The results showed that all patients had a new nonenhancing area in the pancreas consistent with tumor necrosis (range, 9.0–60.0 cm$^3$), and the median survival time after PDT was 9.5 months (range, 4–30). PDT may be valuable for treating localized cancers in patients who are poor candidates for definitive surgery or in whom the location of the tumor makes pancreatic resection inappropriate. Abulafi et al. [79] and Tseng et al. [80] indicated that patients with pancreatic and ampullary carcinoma for whom surgery is not appropriate should be treated with PDT, which is both feasible and safe for small tumors.

Verteporfin, a derivative of a benzoporphyrin, has been proposed for the treatment of PC due to its short metabolic half-life, excitation by near infrared wavelengths and clinical approval for PDT [81]. Only 3 verteporfin PDT studies for PC have been undertaken. Ayaru et al. [82] concluded that the safety profile of verteporfin is very similar to mTHPC, with the advantages of a shorter drug-light interval and drug elimination time. Yusuf et al. [83] showed that endoscopic
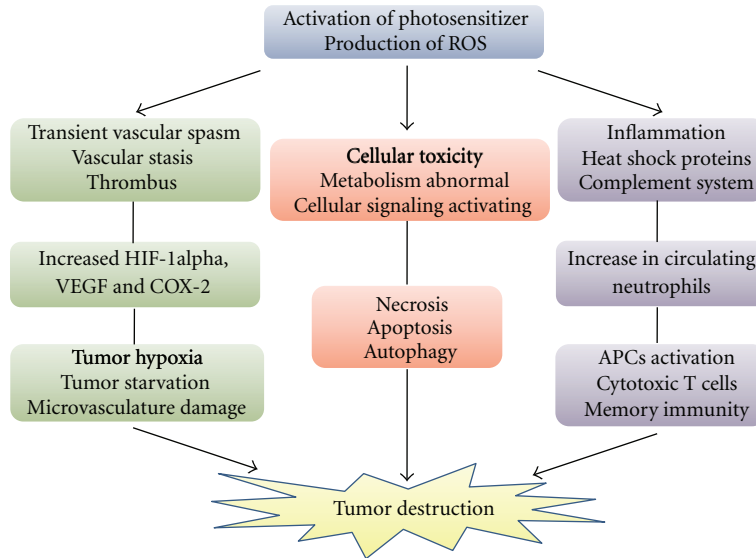
FIGURE 5: Pathways of PDT-induced pancreatic tumor cell death or destruction. The antitumor effects of PDT include three main mechanisms: direct tumor cell killing, vascular destruction, and immune system activation.

ultrasound- (EUS-) guided PDT of porcine pancreas with verteporfin led to pancreatic tissue damage. In different orthotopic PC xenograft models, Samkoe et al. [21] reported that both Panc-1 tumor and AsPC-1 tumor cells were killed by treatment with verteporfin PDT. Verteporfin PDT is now entering a Phase I/II clinical trial at the University College London Hospital [82].

Surgery remains the primary method of treatment for malignancies. However, more than 80% of PC patients have locally advanced or metastatic disease and thus are not amenable for resection at the time of diagnosis [3]. PDT has great potential when combined with surgical resection in the eradication of residual malignant tissues [84]. Surgical resection can clean the tumor bed within the obvious delineation while PDT may destroy the peripheral tissue and in turn enhance the efficiency of cancer removal [85].

PDT has many advantages, including its selective effect on malignant cancer cells of the pancreas versus normal tissue [18]. The precise reason for this phenomenon is still unclear, but it has been assumed to be related to an immuno-logic reaction [86]. Another advantage is that PDT does not lead to the accumulation of toxicity in patients [7, 87]. Furthermore, the combination of PDT with chemotherapy and other treatments can lead to significant additive benefits [22]. However, PDT also has some disadvantages. Side effects such as gastrointestinal bleeding and duodenal obstruction [78] have been noted. In addition, large tumor masses prevent PDT from penetrating the full depth of the cancerous tissue and thus diminish its efficiency [88].

## 4. Conclusions and Future Perspectives

PC remains one of the most devastating neoplasms of the gastrointestinal tract. New therapeutic tools for PC are urgently needed. It is generally believed that the most common therapies for cancer, such as surgery, chemotherapy, and ionizing radiation, are accompanied by immuno-suppression. However, PDT does not share this characteristic and thus presents an attractive alternative to these therapies [65]. PDT in combination with surgery, radiotherapy, chemotherapy, or antiangiogenic therapy has become a subject of research in recent years. This strategy still faces challenges, such as the reduction of side effects and the optimization of the method of treatment (i.e., multiple interstitial optical fibers to increase treated tumor volume), but it may become a superior method for treating PC. Another way to improve PDT is through the development of new photosensitizers. Well-designed experimental studies and clinical studies will be needed for further improving PDT.

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