Review

Tumor Biology

Tumor Biology
October 2017: 1–17

DOI: 10.1177/1010428317734691
journals.sagepub.com/home/tub
SAGE

Targeted photodynamic therapy as potential treatment modality for the eradication of colon cancer and colon cancer stem cells

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Abstract

Colorectal cancer is commonly treated by tumour resection, as chemotherapy and radiation have proven to be less effective, especially if the tumour has metastasized. Resistance to therapies occurs in almost all patients with colorectal cancer, especially in those with metastatic tumours. Cancer stem cells have the ability to self-renew, and their slow rate of cycling enhances resistance to treatment and increases the likelihood of tumour recurrence. Most metastatic tumours are unable to be surgically removed, thus creating a need for treatment modalities that target cancers directly and destroy cancer stem cells. Photodynamic therapy involves a photosensitizer that when exposed to a light source of a particular wavelength becomes excited and produces a form of oxygen that kills cancer cells. Photodynamic therapy is currently being investigated as a treatment modality for colorectal cancer, and new studies are exploring enhancing photodynamic therapy efficacy with the aid of drug carriers and immune conjugates. These modifications could prove effective in targeting cancer stem cells that are thought to be resistant to photodynamic therapy. In order for photodynamic therapy to be an effective treatment in colorectal cancer, it requires treatment of both primary tumours and the metastatic secondary disease that is caused by colon cancer stem cells. This review focuses on current photodynamic therapy treatments available for colorectal cancer and highlights proposed actively targeted photosynthetic drug uptake mechanisms specifically mediated towards colon cancer stem cells, as well as identify the gaps in research which need to be investigated in order to develop a combinative targeted photodynamic therapy regime that can effectively control colorectal cancer primary and metastatic tumour growth by eliminating colon cancer stem cells.

Keywords

Colon cancer, colon cancer stem cells, photodynamic therapy, active targeting, passive targeting

Date received: 17 August 2017; accepted: 7 September 2017

The dreaded colorectal cancer

There are over a million new cases of colorectal cancer (CRC) being diagnosed each year, worldwide. CRC is known to be the third most frequent malignancy and the fourth most frequent cause of cancer-related deaths.[1] Development of CRC) is often attributed to a combination of genetic predisposition and environmental factors. Up to 25% of cases are found to be hereditary, and the remaining cases are due to environmental factors.[2,3] Inherited syndromes include familial adenomatous polyposis (FAP), hereditary non-polyposis colorectal cancers (HNPCC) and other types of tumour with a familial history.[2] Environmental factors that may have an effect are diet low in fibre and high in fat and red meat, heavy alcohol consumption, a sedentary occupation, low physical activity, obesity and cigarette smoking.[4]

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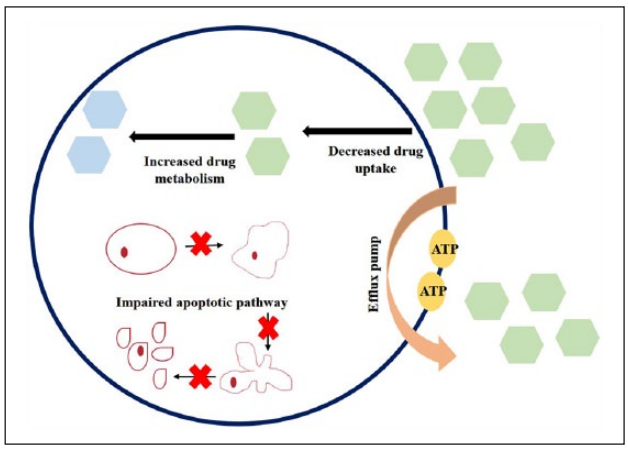

Figure 1. Illustration of mechanisms of tumour cell resistance to therapy. Decreased uptake of chemotherapeutic drugs caused by drug efflux proteins in the cell membrane and changes in drug metabolism. Other metabolic associated resistance occurs through increased aerobic glycolysis, fatty acid synthesis and glutamine metabolism, which results in decreased drug-induced apoptosis.

Most CRCs are thought to develop from adenomatous polyps (growths) arising from the lining of the intestine, and evidence is suggestive that adenomas are possibly present for several years before malignancy progresses. The number and size of adenomas, in addition to their histological type accompanied by the presence of epithelial dysplasia, is thought to affect the risk of development to CRC.[5,6] Regardless of diagnostic and therapeutic advances, tumour recurrence and metastasis are two critical factors effecting the survival rates of patients with CRC.[7] Nearly 50% of patients with CRC will develop metastases, either at presentation or during follow-up.[8]

Pitfalls of conventional treatment methods for CRC

Common treatment modalities for CRC include the following: chemotherapy, radiation therapy (RT), targeted therapy and surgery.[9–11] The treatment of choice is solely dependent on the stage of the disease. At present, the primary treatment method is surgical resection. In early stages of the disease (stage 0 or I), surgical excision is used without the need for further treatment options; however, cancer recurrence is common as it is estimated that one-half of patients will experience a recurrence in the first 3 years post surgery. For Stages I–III, the 'gold standard' therapeutic choice is colon cancer resection along with a proper lymphadenectomy.[12] Patients with stage IV disease require chemotherapy or targeted therapies combined with surgery.[10]

However, to date, chemotherapy only results in objective responses in 30% of cases,[13] and if metastases has occurred, chemotherapy may not be a cure, but does help in improving prognosis, tumour shrinkage and relief of symptoms.[10] RT is usually useful to treat Stage II or Stage III CRC and may shrink unresectable tumours so that they can be surgically removed.[11] RT can also be used to help control spread to other parts of the body in patients that are not healthy enough for surgery.[14] Unfortunately, RT has side effects including nausea, stool leakage, fatigue, sexual problems, skin irritation, rectal irritation and diarrhea.[15] In addition, CRC survivors are at increased risk of second primary cancers of the colon and rectum, as well as other cancer sites.[16]

Resistance to treatment and tumour metastases

The decrease in efficacy of current therapies is due to patients developing resistance. Resistance to therapies occurs in almost all patients with CRC.[9] Several studies have shown drug resistance attributed to mutations and the corresponding deregulated signalling pathways in colon cancer patients. These mutations occur in a group of oncogenes that result in poor responses to targeted therapies.[17] Failure of response to therapeutic drugs occurs in 90% of metastatic CRCs and is attributed to resistance related to an increased aerobic glycolysis, fatty acid synthesis and glutamine metabolism, which consequently leads to a decreased drug-induced apoptosis. Additionally, drug efflux transporter proteins are found to be overexpressed which leads to decreased delivery of the drug to cancer cells, by reduced uptake by the cell or by changes in enzymes involved in metabolism, as shown in Figure 1.[7,18]

RT is often used prior to, or following, surgical resection and often combined with chemotherapy, to reduce the risk of tumour recurrence. A proportion of tumours initially respond well to radiation, but a large proportion of patients experience resistance to RT.[19] When cancer cells exposed to RT prompted DNA damage, the kinase Ataxia Telangiectasia Mutated (ATM) is triggered to stimulate DNA repair pathways. In addition, ATM also controls the pro-survival and radio-resistance pathways in exposed cells.[20] The anti-apoptotic factors phosphatase of regenerating liver-3 (PRL-3) and survivin have also been identified as resistance factors in RT, as these factors have been shown to correlate with advanced CRC, liver and lymph node metastases, high risk of recurrences and shorter patient survival.[21]

In addition to therapeutic resistance, metastasis of CRC is another concern. Mutations in the TGF-β, PIK3CA and TP53 genes are responsible for clonal expansion of a carcinoma, as well cellular potential for invasiveness and metastasis. Metastatic potential is also acquired rapidly by these cells and frequently occurs in the liver. Although it is known that a primary colorectal tumour occurs due to mutations, the molecular basis for the advance of metastatic CRC remains largely unknown.[22]

Another factor that plays an important role in resistance to therapy, as well as tumour metastasis, in CRC is the presence of cancer stem cells (CSCs). CSCs undergo a slow rate of cycling which enhances resistance to treatment (chemotherapy and radiotherapy) and increases the likelihood of tumour recurrence. Additionally, CSCs have the ability to initiate new tumours which may be of important in metastatic colonization.[7]

The controversial CSCs

Origin

Normal adult stem cells (ASCs) have the potential for unlimited replicative abilities and self-renewal capacity. This lead to the hypothesis that stem cells may be the origin for many cancers.[23] The stem cell hypothesis consists of two related, but separate, components: (1) concerns the cellular origin of tumours and (2) that tumours are driven by cellular constituents that display 'stem cell' properties.[24] This subpopulation of cells are presumed to be a result of somatic mutations of a normal ASC, giving it a proliferative advantage, resulting in generation of clonal outgrowth in the tissue ultimately leading to the formation of a neoplasm.[25]

CSCs share the same characteristics as those of normal stem cells, which include ability for self-renewal and differentiation.[26] CSCs have the ability to reconstitute tumours, and proliferate slowly, for an extended period of time.[27] The combination of these mutation/proliferation mechanisms and the tumour microenvironment leads to the different stages of cancer progression.[28] CSCs are also thought to be the root of resistance to conventional treatment methods such as chemotherapy and radiotherapy.[29] Their resistance is a result of their ability to better repair intracellular damage (i.e. DNA and protein damage), and they can effectively quench intracellular reactive oxygen species (ROS), reducing the amount of damage that they incur when under stressful conditions. In addition, CSCs have been identified as the cells responsible for metastases of the primary tumour, survival in the bloodstream and colonization in distant organs.[30]

Evidence of CSCs

The connection between cancer and CSCs was first discovered in the 19th century by histological examination of tumour and embryonic tissue that displayed similarities.[31] A number of studies followed leading up to a discovery in 1960 by Nowell[32] a Hungerford of the Philadelphia (Ph) chromosome and its association with chronic myeloid leukaemia (CML). The succeeding demonstration of the Ph chromosome in all the major non-lymphoid lineages of blood cells from patients with chronic phase CML confirmed Dameshek's prophecy of a possible common origin of the CML clone from a transformed, but still multipotent, haematopoietic stem cell.[15,30] Another study investigating multiple myeloma also presented that only a small subgroup of cancer cells were capable of extensive proliferation.[33]

In 2003, the CSC concept was applied the first time to solid tumours by Clarke et al. These researchers identified a subpopulation of specific marker expressing breast CSCs as the only tumour-initiating population that was able to produce new tumours by serial passaging in immunodeficient mice.[34] Since, CSCs have been isolated from various cancers, including breast cancer,[35] prostate cancer,[36] pancreatic cancer,[37] head and neck cancer,[38] lung cancer,[39] hepatocellular carcinoma,[40] and renal CSCs.[41]

Identifying CRC stem cells

CSCs are identified by the expression of specific markers, and several markers have been proposed in CRC.[42] Colon cancer stem cells (CCSCs) were first identified in 2007 by two different research groups using CD133.[43,44] The study revealed that only a small subset of CD133+ cells was capable of initiating tumour growth, while negative cells were not, and although normal colon cells expressed CD133, they did so at lower numbers.[45] Similarly, CD44-positive cells showed a higher capacity to form clones in vitro and to generate xenograft tumours in immunodeficient mice.[46]

Levin et al.[47] exhibited that CD166 marks the stem cell function in the intestinal crypt in both mice and humans. This is suggestive of CD166-expressing cells importance in the establishment and maintenance of the endogenous intestinal stem cell niche. The three above-mentioned cell surface markers are the main markers currently being associated with CRC stem cells. Although findings from studies using these markers separately are controversial, their combined analysis may be effective in identification of low, intermediate and high-risk cases of CRC.[48] Additional markers include those that were found to be associated with stemness characteristics.[45] A summary of the potential CRC stem cell markers and their functions are given in Table 1.

Current eradication methods of CSCs

Failure to eliminate CSCs is believed to be an underlying cause for resistance to conventional therapy and recurrence of malignancy. In addition, continued use of conventional chemotherapeutics is associated with added toxicities, which can be fatal. It is, therefore, necessary to implement therapeutic strategies that specifically target colon CSCs. Yu et al.[57] studied the effects of combining a conventional colon cancer chemotherapeutic regimen with curcumin and found that it could be effective in reducing/eliminating the CSCs. The HOXA5 protein is an important

Table 1. Cancer stem cell markers in colorectal cancer.

Marker	Function	Reference
CD133	Transmembrane glycoprotein	Shmelkov et al.[49]
CD 29	Integrin that mediates cell–ECM adhesion and is involved in homing to sites of inflammation	Vermeulen et al.[50]
CD44	Cell surface glycoprotein involved in cell adhesion and migration	Du et al.[46]
CD24	Cell-adhesion molecule	Shmelkov et al.[49]
CD26	Cell surface glycoprotein with intrinsic dipeptidyl peptidase IV activity with a significant role in tumour pathogenesis and progression	Hofving[51]
CD166	Involved in neuronal extension, embryonic haemopoiesis and embryonic angiogenesis	Dalerba et al.[52]
EpCAM	Cell-adhesion molecule	Dalerba et al.[52]
ALDH1	Detoxifying enzyme responsible for the oxidation of intracellular aldehydes	Huang et al.[53]
Msi-1	RNA-binding protein	Khalek et al.[54]
Wnt activity/β-catenin	Protein in Wnt/β-catenin pathway	Jiang et al.[55]
Lgr5	G protein-coupled receptor gene encoding for a component of the Wnt receptor complex	Baker et al.[56]

ECM: extracellular matrix.

repressor of intestinal stem cell fate in vivo. In colon cancer, the HOXA5 protein is downregulated, and its re-expression results in loss of the CSC phenotype, averting tumour advancement and metastasis. Retinoids can be used to trigger tumour regression by HOXA5 induction and offer a means to treat colon cancer by eradicating CSCs.[58]

Other forms of CSC–targeted therapies include monoclonal antibodies, blockage of self-renewal pathways by small molecular inhibitors, and induction of differentiation and the disruption of epithelial–mesenchymal transition (EMT). Resistance of CCSCs to the drug oxaliplatin can be overcome by sensitizing cells with an interleukin-4 blocking antibody which effects their stemness and drug resistant properties. Other studies have testified the efficacy of anti-EREG antibody (epiregulin, epidermal growth factor family) against tumour metastasis in a metastatic model tested suggesting that the anti-EREG antibody is successful in the early stage of cancer expansion when cancers are rich in CSCs.[7]

Targeting and inhibiting the small molecules involved in stem cell pathways can also be effective treatment strategies. An example is the Wnt proteins, which are cysteine-rich molecules, that play a critical role in the development of various organisms and a vital role in embryogenesis and propagation, survival and differentiation of haematopoietic stem cells.[59] Defects in the Wnt/β-catenin signalling pathway have been implicated in several types of human cancers, including ovarian colon cancer, and also play a critical role in CSCs. Stem cells with high levels of Wnt/β-catenin signalling display greater tumourigenic potential and, therefore, targeting the Wnt/β-catenin signalling pathway could be a potential treatment for CCSCs.[60]

The gene p53 induces cell-cycle arrest, senescence or apoptosis preventing the build-up of genetic mutations within cells undergoing stress. The gene is mutated in several human cancers, including colon cancer, and cancer progression is reliant on loss of WT p53 function.[61,62] Restoration of the WT p53 function is essential for the efficacy of chemotherapy and radiation, thus p53 restoration compounds may be used to enhance chemo- and radiosensitivity.[63] p53 also plays a role in the suppression of factors involved in the maintenance of self-renewal of mesenchymal stem cells (MSCs). Growing evidence supports that deregulation of the functions of embryonic stem cells (ESCs), which could be due to p53 mutations, and ASCs may lead to developmental abnormalities, alterations in adult tissue maintenance and generation of CSCs.[64] Restoration of WT p53 gene activity could be a promising tumour-specific regimen for targeting the CSC population.[61]

Targeted agents, as shown in Figure 2, have been established and have demonstrated improved outcome in metastatic CRC patients, in combination with chemotherapy.[10] However, although these therapies have shown promise, anti-angiogenic drugs have proven to be toxic and affect multiple organs, and cancers have shown to become resistant to small-molecule inhibitors. None of the above-mentioned targeted therapies have shown to be a cure.[65,66]

Photodynamic therapy an emerging treatment modality

Photodynamic therapy (PDT) is a promising method used for the control of a variety of cancers.[67] PDT is a harmonized process which first requires the exposure of the cancer tissue to a photosensitizer (PS), administered either topically or intravenously, depending on the location of the targeted tissues.[68] A PS is a molecule that is taken up and localizes in the target cell and/or tissue and can only be activated by light.[69] Activation of a PS is achieved through exposure to laser irradiation at a specific wavelength. Once

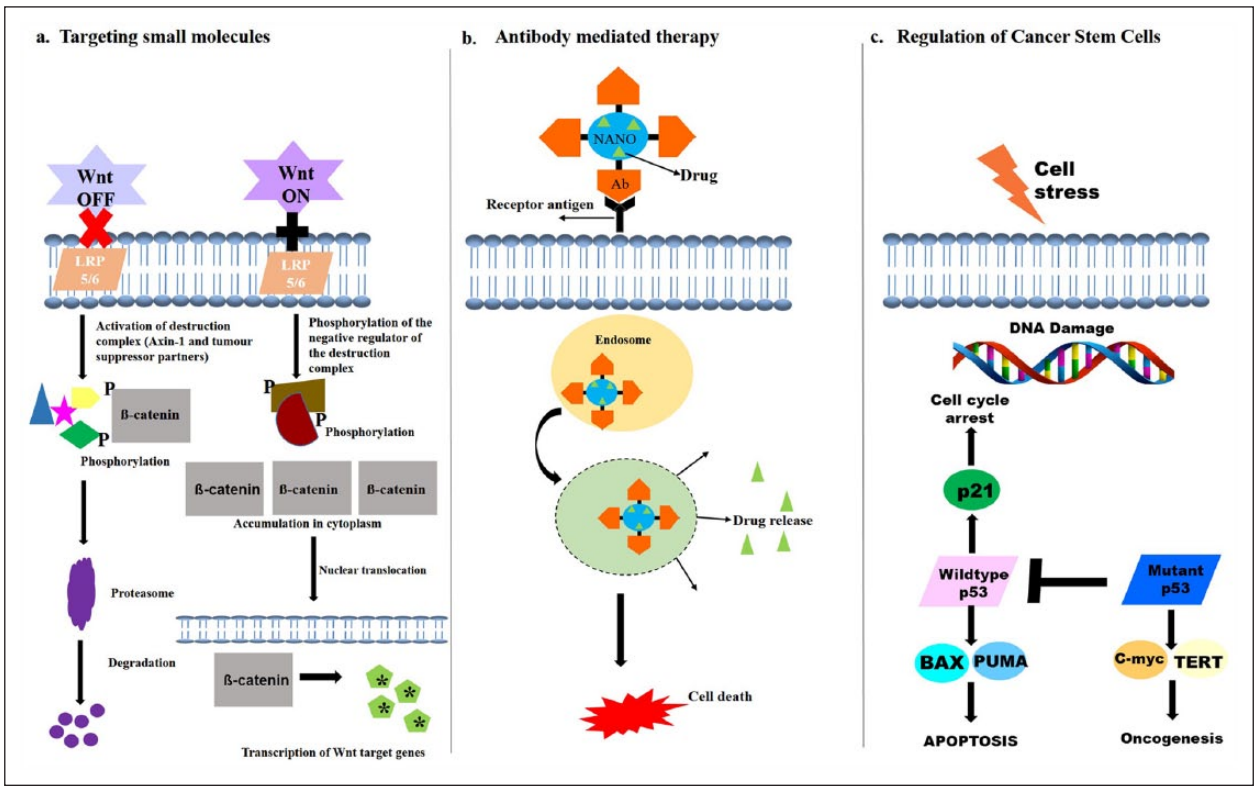

Figure 2. Diagram representing the current methods in cancer stem cell treatment. (a) The effects of the Wnt pathway when activated or not activated. Defects in the Wnt/β-catenin signalling pathway plays a critical role in CSCs. Stem cells with high levels of Wnt/β-catenin signalling have greater tumourigenic potential. Targeting the Wnt/β-catenin signalling pathway could be a potential treatment for CCSCs. (b) A chemotherapeutic drug combined with an antibody used for cancer therapy. Using antibody-mediated therapies could specifically target cancer stem cells and enhance drug delivery and subsequent cell death. (c) The role of EMT gene p53 and its role in cancer therapy. Restoring functional p53 gene could be a promising tumour-specific regimen for targeting the CSC population.

photons are absorbed by a PS, it is excited and stimulated from the ground state to a higher level of energy, a singlet state.[70] Alternatively, the molecule may convert to the triplet state through a mechanism called intersystem crossing, which results in a change in the spin of an electron. In this triplet state, the PS reacts with molecular oxygen and gives rise to free ROS that can destroy cancer tissue[71] (Figure 3).

Efficiency of PDT depends on the production of ROS in the cell that are generated through two types of photoreactions. In Type I, the PS reacts with biomolecules, through a hydrogen atom (electron) transfer, to form radicals which react with molecular oxygen to generate ROS, subsequently leading to the production of oxidative stress and ultimately cell death. In the second reaction type (Type II), energy is transferred directly to oxygen in the cell to form a singlet oxygen (a subset of ROS) which then oxidizes various substrates resulting in cell death.[72]

A major advantage of using PDT is that it achieves selective cell destruction and minimizes damage to adjacent healthy structures. PSs are taken up by all cells; however, they tend to preferentially localize in diseased tissue and remain in diseased tissue for a longer period of time due to the enhanced permeability retention (EPR) effect.[73] Consequently, it is vital to ensure PS activation only occurs once the proportion of PS in diseased tissue is greater than that present in healthy tissue.[74] Other advantages of PDT over conventional treatment options include being a minimally invasive technique, lowering morbidity rate, ability to reserve the anatomic and functional integrity of many cells, minimal side effects, selective targeting, and no drug resistance, as well as reduced toxicity which allows for repeated treatment.[75]

Overcoming resistance to PDT of CSCs

CSCs are thought to be resistant to conventional cancer therapies, including PDT, but resistance to PDT depends on a variety of factors. Figure 4 shows examples of overcoming resistance to PDT.

Subcellular localization of PSs

PS uptake and localization play a critical role in the effectiveness of PDT in the treatment of cancer. Subcellular localization of photosensitizers in different

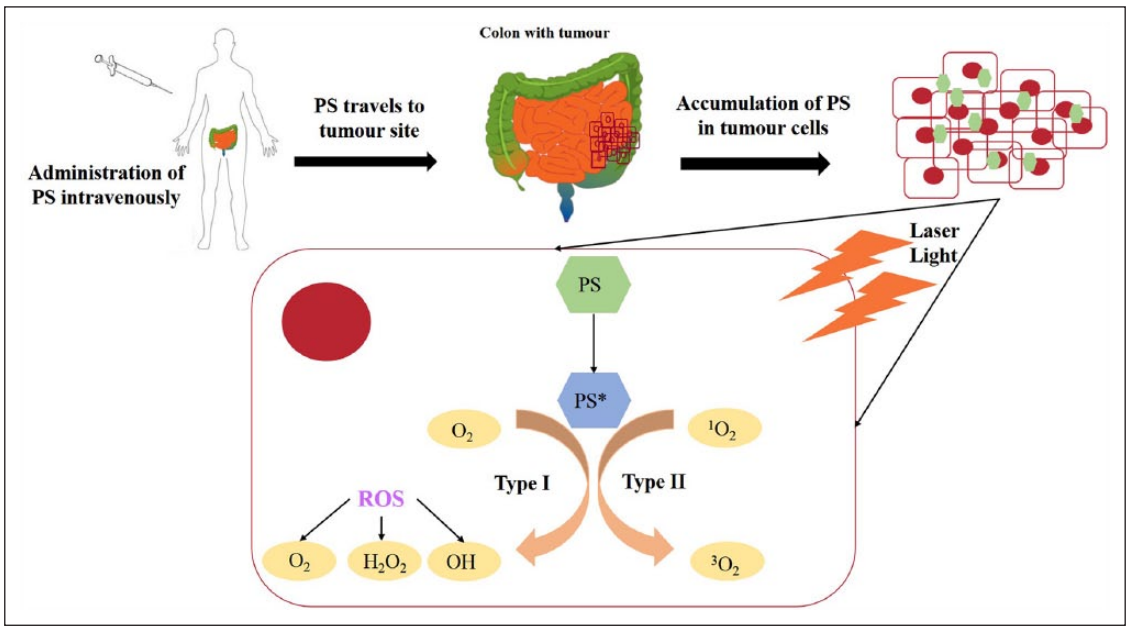

Figure 3. Diagram representing photodynamic treatment of cancer. Photodynamic therapy is initiated by injecting a photosensitizer into a patient, transport of the photosensitizer to the tumour site through the bloodstream, and uptake of the PS by the tumour cells. Once the PS is localized in the tumour cells, laser light is applied to the site, penetrates through the skin and excites the PS. The PS then undergoes either a type I or type II photoreaction producing either reactive oxygen species or a singlet oxygen, both capable of inducing cell death.

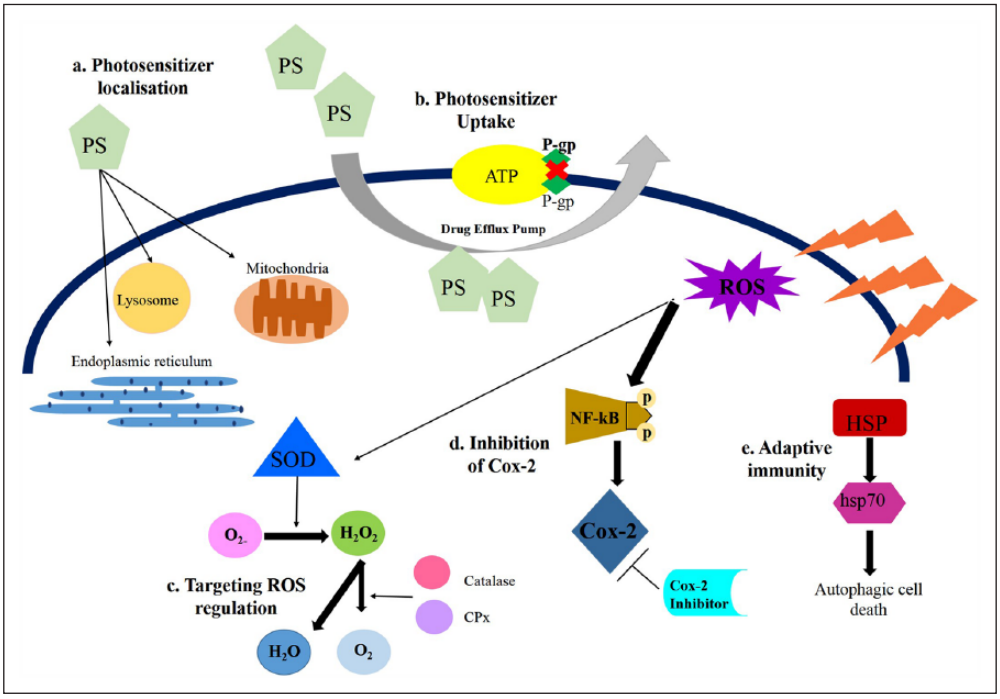

Figure 4. (a) Photosensitizer localization – subcellular localization of PSs in different cellular components induce various pathways of cell death/damage. PSs that localize in mitochondria are commonly used as after illumination they lead to apoptosis of the cell. (b) Photosensitizer uptake – photodynamic resistance may be due to genes that inhibit the transport of the PS into the cell. Altering the expression of these proteins by administering blockers in conjunction with PDT, PSs may overcome cellular resistance. (c) Targeting ROS – superoxide dismutase (SOD) is an essential antioxidant enzyme that defends cells against potentially damaging superoxide radicals. Suing PDT in combination with SOD inhibitors may increase efficacy. (d) Inhibition of Cox-2 – COX-inhibitors can be used in combination therapy to increase success in overcoming tumour immune evasion. (e) Adaptive – immunity PDT can lead to the redistribution of HSPs on the cellular surface and enhance the development of adaptive immunity towards the cancer cell.

cellular components may induce various pathways of cell death/damage. Subcellular localization sites of PSs include the plasma membrane, lysosomes, Golgi apparatus, the nucleus and the mitochondria.[76] PSs localizing in lysosomes can lead to cell killing upon illumination, but the relative efficacy is significantly lower when compared to a PS localized in the mitochondria and other organelles. In mitochondria, many PSs cause mitochondrial damage after illumination and subsequently lead to apoptosis of the cell, this is, therefore, the most common type of PSs used. PSs that accumulate in smaller amounts in more than one organelle (co-localization) may be used in combination to enhance the PDT efficacy of the PSs.[77]

PS solubility

Solubility also plays a role as most PSs are hydrophobic.[76] Hydrophobicity and a tendency to aggregate in aqueous environments hinder bioavailability of several PSs. Aggregation reduces increased uptake of photosensitization by the mononuclear phagocytic system (MPS) and decreased uptake by target cells as well as an increased risk of anaphylactic reactions.[78] Sulphonation of PSs can aid in overcoming these issues as it affects the lipophilicity of a drug; henceforth, its cellular uptake and tendency to aggregate in cells may also play a role in the tumour localizing ability of a drug. Phthalocyanine PSs (PCSs) can be tuned for optimal solubility and minimal aggregation by the introduction of substituents in the peripheral positions of the tetraaza isoindole macrocycle, coordination of metal ions with the central atoms, and the addition of axial ligands in the fifth and sixth positions. Another modification could be the conjugation to a nano-particle (NP) which could increase solubility and cellular uptake. Such modifications can tune the water solubility and aggregation of the PCSs, without significantly affecting its photophysical properties.[79]

PS delivery and selectivity

Abundant literature describes the use of NPs as a delivery system of drugs to increase the response to anticancer compounds.[80] A wide variety of organic and inorganic nano-constructs, such as liposomal, micellar, polymeric, silica and gold NPs, have been introduced to deliver high payloads of PS to desired sites, when combined with targeting processes.[81] Advantages of using NPs include lower levels of the PSs used in treatment, increased selectivity, reduced side effects and reduced dark toxicity. In addition, peptide or antibody tags in NP systems can increase selectivity more efficiently and aid in controlling the size of the particle, which can influence better passive targeting through EPR effect and, therefore, increased cellular uptake.[82]

Antibody-mediated specificity

In an effort to increase PS accumulation specificity and reduce unwanted PDT PS and NP side effects, significant effort has been devoted towards the synthesis, and characterization, of bio-conjugates. Synthesis with either NPs or PSs further enhances PDT NP-PS passive drug delivery by actively and specifically targeting tumourous cells with monoclonal antibody (mAb) conjugates. In the case of anticancer-mediated PDT, malignant cells present different types, as well as greater amounts, of many surface antigens.[83] Antibodies against tumour-associated antigens are easily generated, and if correctly attached to a PS drug delivery system, the PS can be directly targeted and absorbed via cell membrane endocytosis into specific tumours and therefore causes targeted cancer cell death upon PDT light activation.[84]

Small-molecule inhibitors

Cells resistant to PDT may express genes that inhibit the transport of the PS into the cell. The main focus has been on P-glycoprotein (P-gp) and adenosine triphosphate (ATP)-binding cassette sub-family G member 2ABCG2 as PDT inhibitors. The expression of P-gp in tumour cells results in a reduction of intracellular drug concentrations and subsequent decrease in cytotoxicity.[85] ABCG2 is an ATP-binding cassette half-transporter overexpressed in cells resistant to several drugs and has been identified in a wide variety of tumours including adenocarcinomas of the digestive tract.[86] As a member of the family of multidrug resistance proteins, it protects cell from exogenous and endogenous toxins through the efflux system.[87] Altering the expression of these proteins by administering blockers in conjunction with PDT, PSs may overcome cellular resistance.

ROS

ROS are the natural by-products of cellular oxidative metabolism. Cells exposed to PDT undergo stress and ROS are created as intermediates, and their cellular levels are controlled by various detoxifying enzymes.[88] Superoxide dismutase (SOD) is a crucial antioxidant enzyme that defends cells against potentially damaging superoxide radicals, like those produced in PDT. In order to overcome the protective effects of SOD, combinations of SOD inhibitors such as; potassium cyanide, chloroform-ethanol, H_2O_2 and NaN_3, administered together with PDT might increase the efficiency of the anticancer treatment.[89,90] Modified PDT techniques have been developed to overcome reduced oxygen levels, which ultimately reduces the efficacy of PDT in tumour microenvironments, which houses CSCs. Usacheva et al.[91] increased

production of ROS using surfactant–polymer NP which also proved effective at eliminating CSCs.

Heat-shock proteins

Heat-shock proteins (HSPs) are reported to provide effective cytoprotection under various stress stimuli.[92] In addition, it regulates inflammatory and immune responses.[93] The most studied being Hsp70 which is overexpressed in cells under heat stress and protect proteins from being damaged.[94] Some PSs used in PDT therapy can induce the expression of HSP70; however, porphyrin-derived PSs have shown to be less effective in the induction of HSP70 expression but leads to redistribution of HSP70 to cell surfaces. HSP70 on the cell surface subsequently facilitates the development of adaptive immunity by providing a specific signal that activates macrophage uptake of apoptotic bodies. Therefore, to increase effectiveness of PDT, combining it with hyperthermic (HPT) treatment could increase the expression of HSP70 and its enhanced surface localization on treated tumour cells.[95] Studies on glioblastoma have shown that the combined HPT and PDT approach is quite effective to treat this type of cancer.[96]

Hypoxia

PDT-induced hypoxia and inflammation lead to changes in the tumour microenvironment associated with increased expression of angiogenic and pro-survival molecules, including cyclooxygenase-2 (COX-2).[97] COX-2 is an inducible form of the enzyme that catalyzes the first step in the synthesis of prostanoids, which is associated with inflammatory diseases and carcinogenesis.[98] COX-inhibitors have proven success in overcoming tumour immune evasion. It has been proposed that COX-inhibitors sensitize type 1 immune responses by inhibiting M2 macrophages, T regulatory cells and myeloid-derived suppressor cells (MDSC) and enhancing dendritic cells (DC), natural killer (NK) and cytotoxic T-lymphocyte functions. Use of selective COX-2 inhibitors could result in a substantial risk reduction in many cancers including CRC. Findings from the study by Rahman et al.[99] presented the potential benefit of combining COX-2 inhibitors with current cancer treatment regimens to achieve better responses.

PSs applied in photodynamic treatment of colon cancer and CCSCs

Tetrapyrollic photosynthetic drugs such as porphyrins, chlorins and phthalocyanines have shown to be effective in PDT of CRC.[100] Table 2 lists some current PDT studies which utilize different types of PSs for the in vitro treatment of CRC and CCSCs.

Targeted PDT for colon cancer and CCSCs

PS targeting of CRC and CRC stem cells

Although some PSs used in PDT reveal certain tumour selectivity by the EPR effect, they can also accumulate in healthy tissues causing side effects such as phototoxic and photoallergic reactions.[117] To avoid this complication, targeted photodynamic therapy (TPDT) was fashioned to improve PS drug delivery to cancer tissue, and the overall specificity and efficiency of PDT was increased.[100] TPDT can be divided into two mechanisms of action: passive or active targeting. Passive PDT targeting makes use of the PSs drug carrier's physicochemical factors, as well as the morphological and physiological differences between normal and tumour tissue (i.e. EPR effect) to deliver the PS to a target site.[73] Active PDT targeting involves PS drug delivery to a specific tumour site, which is based on a molecular recognition process, using specific ligands or antibodies which bind to overexpressed cancer cell receptors.[100] These two cellular uptake mechanisms have been illustrated in Figure 5.

Passive targeting of CRC and CRC stem cells

There is a great interest in NPs as drug carriers for selective transporting of PSs to CRC and CCSC cells.[7,100] Most PS drug delivery systems are optimized with NPs to enhance passive drug uptake, promote solubility and stability and limit non-specific toxicity.[118] Examples of nano-carrier platforms used to assist in the co-delivery of drugs for CRC therapy include liposomes, polymers, micelles, dendrimers, silica, nano-emulsion, nano-tubes and nano-gels.[119] These types of NPs, especially polymeric NPs, have the advantage of protecting PS drugs from chemical and enzymatic degradation in the gastrointestinal tract and therefore increase their stability and absorption across the intestinal epithelium with controlled drug release.[117,120] Various studies have been conducted investigating the effective drug delivery of PS to CRC and CCSCs utilizing nano-drug carriers (Table 3).

Active targeting of CRC and CRC stem cells

To improve uptake of PSs in CRC and CCS cells, active targeting has also been developed. This involves a PS that is directly delivered to the target site using specific ligands or antibodies which bind to overexpressed CRC cell receptors[7,100,131] (Figure 6).

Recently, several active drug-carrying and cellular target systems have been investigated (Table 4). Most PS drug constructs consist of PS drug conjugations to nanocarriers which are further functionalized with monoclonal antibodies (mAbs), antibody constructs or small-molecule inhibitors. These are specifically directed

Table 2. Current PDT studies which utilize different types of PSs for the in vitro treatment of CRC.

Colorectal cancer cells		
Photosensitizer	Remarks	Ref.
3,4,5-trimethoxyphenyl, 3-hydroxyphenyl,4-hydroxyphenyl and sulfonamide phenyl porphyrin derivatives	In CRC cell line, HCT-116 PS porphyrin derivatives induced significant apoptotic cell death	Banfi et al.[101]
5,15-diaryltetrapyrrole derivatives porphyrin derivatives	In CRC cell line, HCT-116 PS porphyrin derivatives induced significant apoptotic cell death and high ROS yields	Gariboldi et al.[102]
Lipophilic 5-aminolevulinic acid	In CRC cell lines SW-480, HT-29 and CaCO-2, PS uptake was enhanced	Brunner et al.[103]
Meta-tetra (hydroxyphenyl) chlorine (mTHPC)	In CRC cell lines Colo-201 and PDT, phototoxicity was noted, PS localized in the liposomes and apoptotic cell death was induced	Leung et al.[104]
Lysosome localizing chlorin e 6 (Ce6) ATX-S10Na(II)	In CRC cell line HCT-116, early apoptosis and cell death induced was induced by Bax- and p53-dependent proteins	Mitsunaga et al.[105]
Pheophorbide – a methyl ester (PPME)	PDT induced phototoxic apoptosis in CRC cell line HT-29	Xu et al.[106]
Sulphonated zinc phthalocyanine ($ZnPcS_{mix}$)	$ZnPcS_{mix}$ localized in multiple organelles in CRC DLD-1 and CaCo-2 and so induced significant apoptotic cell death in PDT applications	Sekhejane et al.[107]
Gallium phthalocyanine	PDT induced cytotoxic effects in CRC cell line CaCO-2	Maduray and Odhav[108]
Glycoconjugated chlorin (H2TFPC-SGlc)	In CRC cell lines MKN28, MKN45, HT-29 and HCT-116, suppressed cell growth and apoptotic cell death were observed after PDT applications	Tanaka et al.[109]
Photofrin II (Ph II) and hypericin (Hyp)	In doxorubicin-sensitive LoVo and doxorubicin-resistant LoVo DX CRC cell line, combination of both PDT and PS reduced multidrug resistance efflux proteins P-glycoprotein (P-gp) and therefore induced a more combination effective cytotoxic cell death	Saczko et al.[110]
Colon cancer stem cells		
Photosensitizer	Remarks	Ref.
Protoporphyrin IX	PpIX-mediated PDT induced autophagy in in vitro and in vivo tumour CSCs and therefore elevated their sensitivity to PDT	Wei et al.[111]
Photofrin	Combination effect of Photofrin PDT as well as chemo and radiotherapy was investigated in 23 young patients with advanced CRC, and PS effectively improved clinical symptoms and reduce complications	Sun et al.[112]
Photofrin	Study investigated Photofrin PDT cytotoxic effect in CRC HT-29 and HT29-P14 cell lines and noted Hsp60 induction which contributed to CCSC resistance to apoptosis	Hanlon et al.[113]
Protoporphyrin IX induced by 5-aminolevulinic acid	Factors which affected the PpIX clearance ratio from WiDr human colon carcinoma cells and ALA drug uptake included PS concentration and application time, cell density, temperature, pH, iron content, intracellular amount and localization	Juzeniene et al.[114]
Chlorin e 6 (Ce6)	Ce6-PDT decreased metastatic gene matrix metalloprotienases (MMPs) and chloride intracellular channel 4 (CLIC4) expression in CRC C26 cells resulting in phenotypic changes and suppressed migration	Li et al.[115]
Hypericin	In CRC cells, HCT8 and HCT-116 hypericin–mediated PDT was found to increase the expression of oxaliplatin (L-OHP) by ROS, which affected drug efflux, GSH-related detoxification and NER-mediated DNA repair and therefore in turn reduced the effectiveness of PDT	Lin et al.[116]

PDT: photodynamic therapy; CRC: colorectal cancer; PS: photosensitizer; ROS: reactive oxygen species.

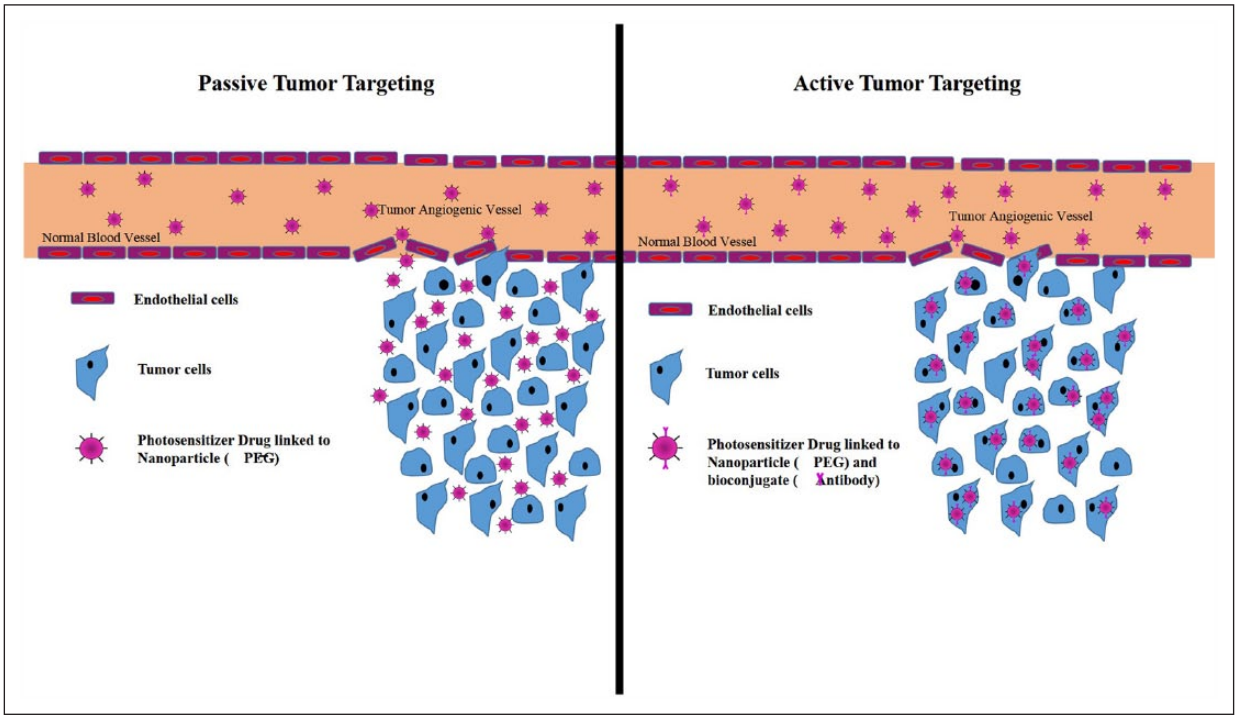

Figure 5. Passive and active tumour targeting mechanisms utilized in targeted photodynamic therapy (TPDT) to enhance cellular uptake of photosynthetic drugs. Passive uptake of PSs involves the EPR effect, while active PS drug targeting involves PS drug delivery to a specific tumour site which is based upon a molecular recognition process.

Table 3. Passive targeting PDT PS drug delivery mechanisms in CRC and CCSCs.

Colorectal cancer cells			
Photosensitizer	Nano-particle	Remarks	Ref.
Meso-tetra (carboxyphenyl) porphyrin (TCPP)	Poly D,L-lactide-co-glycolide (PLGA)	Rapid endocytosis internalization and uptake of TCCP, with enhanced phototoxicity in SW480CRC in vitro cells was due to nano-drug carrier. In vivo tumour growth inhibition experiments in 4-week-old female athymic mice noted that TCPP NPs plus PDT treatment induced the most significant tumour inhibition	Hu et al.[9]
5-aminolevulinic acid (ALA)	Chitosan	Nano-drug carrier remained stable, without aggregation, and showed enhanced cellular absorption in Caco-2CRC cells	Yang et al.[121]
Protoporphyrin IX (PpIX)	Non-biodegradable silica	Nano-drug carrier reported enhanced PS accumulation in both HCT-116 cell lines and tumour bearing mice, with improved ROS generation	Simon et al.[122]
Meta-tetra (hydroxyphenyl) chlorine (mTHPC)	Liposomal formulation FosPeg®	In PDT application in HT-29 cell lines, enhanced phototoxicity and cell death was observed, due to improved PS absorption	Wu et al.[123]
SN-38-cyclodextrin complexation	Chlorin-core star-shaped block copolymer (CSBC) micelles	The synergistic combination of PDT and chemotherapy was effective for the treatment of HT-29 human CRC xenograft model cells as this drug combination inhibited tumour growth with 60% complete regression after three treatments	Peng et al.[124]
Curcumin and 5-fluorouracil	Chitosan	Combinatorial anticancer effects of drug molecular system towards colon cancer HT-29 cell line had a three-fold increase in anticancer effects	Anitha et al.[125]
5-Aminolevulinic acid	Copolymer methoxy poly(ethylene glycol)-chitosan	Superior delivery and PDT phototoxicity	Lee et al.[126]

(continued)

Table 3. (Continued)

Colon cancer stem cells

Photosensitizer	Nano-particle	Remarks	Ref.
Oxaliplatin	Chitosan micelles	Oxaliplatin-incorporated micelles eliminated cancer stem cell and bulk cell populations in CRC tumour cells both in vivo and in vitro	Wang et al.[92]
5-Flurouraci (5-FU)	Solid lipid	5-Flurouracil solid-lipid Nano-particles with in vitro PDT eliminated CCSCs due to enhanced drug delivery and cell membrane penetration of these chemo-resistant tumours.	Yassin et al.[127]
None	Biodegradable lipid nano-carriers containing MDR1-directed siRNA	Novel biodegradable lipid nano-complex for siRNA delivery significantly improved the chemosensitivity in human CCSCs to paclitaxel, as siRNA mediated the knockdown of the drug efflux protein MDR1 that is overexpressed in CCSCs	Liu et al.[98]
None	Silver-based nano-particles	Silver-based nano-particles induced apoptosis in human CCSCs (HCT-116) that have p53 expression	Satapathy et al.[128]
None	Lipid nano-carrier	Lipid nano-complex with siRNA mediated showed knockdown of the drug efflux protein MDR1 that is expressed in CCSCs	Fischer et al.[129]
Porfimer sodium (PII) and 2-(1-hexyloxyethyl)-2-devinylpyropheophorbide-a (HPPH)	None	Two-step tumour and immune controlling PDT regime, which enhanced anti-tumour immunity and controlled metastatic tumour growth in murine colon 26-HA cells	Shams et al.[130]

PDT: photodynamic therapy; CRC: colorectal cancer; PS: photosensitizer; ROS: reactive oxygen species; CCSC: colon cancer stem cell.

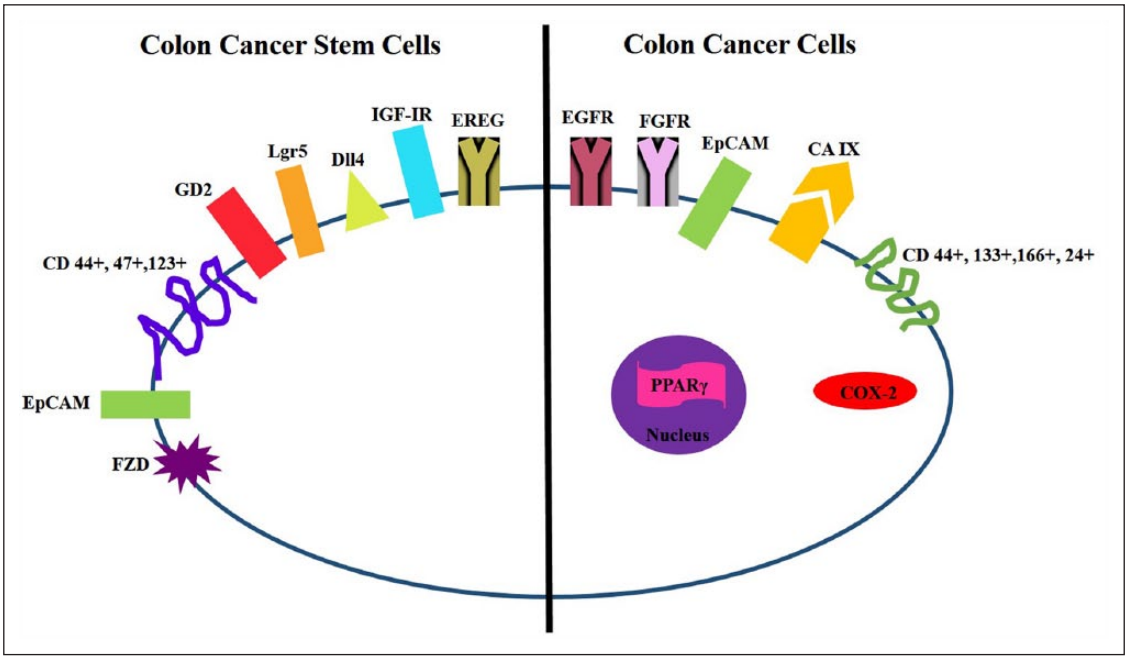

Figure 6. Common proteins that are overexpressed in CRC cells that are possible targets for drug treatment. They include epidermal growth factor receptor (EGFR), fibroblast growth factor receptor (FGFR), epithelial cell-adhesion molecule (EpCAM), carbonic anhydrase IX (CA IX), peroxisome proliferator-activated receptor γ (PPARγ), cyclooxygenase-2 (COX-2), as well as cluster of differentiation 44, 133, 166 and 24 (CD44+, CD 133+, CD166+ and CD24+).[13,132,133] Additionally, technologies such as antibody or NP PEGylation, polysialylation and albumin inclusion have been used to engineer specific active targeting PS drugs.[132] Figure 5 also indicates examples of monoclonal antibodies (mAbs) and antibody constructs that are directed against CCSC-specific surface molecules. These include CD44+, CD47+, CD123+, EpCAM, ganglioside receptor 2 (GD2), leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5), insulin-like growth factor I receptor (IGF-IR), delta-like ligand 4 (Dll4), frizzled (FZD) and epiregulin (EREG) receptors.[7,134] These surface molecules have the potential to be utilized as active targeting sites for specific PS drug delivery mechanisms in CCSCs and overall PDT enhancement in possibly preventing secondary CRC metastasis.

Table 4. Active targeting PDT PS drug delivery mechanisms in CRC and CCSCs.

Colon cancer			
Photosensitizer	Active drug delivery system	Remarks	Ref.
Verteporfin succinimidyl ester	Single-chain variable fragments (scFvs), antibody fragments	Photoimmune conjugate retained photophysical functionality and drug delivery mechanism was more potent than free PS. In in vitro and in vivo PDT applications, it effectively killed tumour LoVo (CEA+, HER2−) cells	Bhatti et al.[135]
Chlorin e6 (Ce6)	Site-specific immune conjugates (murine monoclonal antibody 17.1A) which recognize antigen expressed in HT-29CRC cells	Photoimmune conjugate had cationic electric charge which enhanced PS delivery and showed a 90% phototoxic effect in vitro	Del Governatore et al.[136]
Chlorin e6 (Ce6)	Phototoxic DNA aptamers were bound to unique short O-glycan-peptide signatures on HT-29CRC cells surfaces	Molecular conjugate reported more than a 500-fold increase in toxicity in PDT, with no cytotoxicity noted in control groups	Ferreira et al.[137]
Meso-tetraphenyl chlorin disulfonate (TPCS2a)	IM7-saporin (immunotoxin targeting CD44 receptor towards WiDr CRC) was transported into cells with the use of photochemical internalization (PCI)	PDT treatment with drug carrier resulted in cytotoxic response with a 90% reduction in cell viability	Bostad et al.[138]
5-Fluorouracil	Eudragit S100 coated citrus pectin nano-particles	Citrus pectin is overexpressed in CRC cells and acts as a ligand for galectin-3 receptors. Eudragit S100 is a pH-responsive enteric polymer. Specific site delivery was observed in in vitro and in vivo studies with controlled drug delivery observed only at target sites and showed enhanced PDT cytotoxic effects	Subudhi et al.[139]
None	Cetuximab-conjugated magneto-fluorescent silica nano-particles	Cetuximab-conjugated magneto-fluorescent silica nano-particles effectively targeted in vivo colon cancer cells via EGFR receptors and uptake was amplified by external magnetic field	Cho et al.[140]
Pyropheophorbid-a (PPa) protoporphyrin	ATP-binding cassette sub-family G2 (ABCG2) porphyrin-based targeted PDT	HT-29 cells show high levels of ABCG2 expression and PS uptake was enhanced by this active targeting drug delivery system, with significant PDT-induced cell damage	Kim et al.[141]
Colon cancer stem cells			
Photosensitizer	Active drug delivery system	Remarks	Ref.
None	Anti-EpCAM/anti-CD3	Human recombinant single-chain bispecific bifunctional mAb construct that has effective targeting abilities	Constantinou et al.[142]
None	Anti-IGF-R	Humanized IgG2 mAB targeting abilities	Li et al.[143]
None	Anti-DLL4	Humanized IgG2 mAB targeting abilities	Dallas et al.[144]
None	Anti-frizzled 1, 2, 5, 7, 8	Humanized IgG2 mAB targeting abilities	Gurney et al.[145]

PDT: photodynamic therapy; CRC: colorectal cancer; PS: photosensitizer; EGFR: epidermal growth factor receptor.

at CRC and CCS cell surface receptors or target key components of the intrinsic signalling pathways of the cells.[146]

Conclusion

Tumour reoccurrence and metastasis is still a major concern among CRC patients. The search for alternative therapies to increase therapeutic effect and target cancer cells directly, as well as eliminate CSCs, is well underway. PDT offers a less invasive and targeted form of therapy against cancer. How to maximally accumulate drug at tumour sites and be able to eliminate CCSs is still the main challenge among PDT researchers.

Numerous studies highlighted within this review have shown the effectiveness of active TPDT in CRC to improve tumour uptake of PSs. Photosynthetic drugs are directly delivered to target sites using nano-platforms bound to specific ligands or antibodies which target overexpressed CRC cell receptors, with limited toxicity to normal tissues. This review has identified limits in the use of active TPDT mechanisms to enhance PS tumour uptake in CCSCs. Hence, PDT active drug delivery systems that specifically target overexpressed proteins in CCSCs need to be investigated in order to treat and prevent secondary metastasis. These PS drug delivery systems will need to be able to be effectively retained in CCSCs, evade immune system components, target both CRC and CCSCs as well as be released when maximum accumulation in target cells is acquired.

Unfortunately, current PDT treatments for CRC use high doses of phototoxic drugs which result in adverse effects to the patient. Therefore, it is necessary to develop new photosynthetic nano-medicines with multifunctional characters that bring together different chemotherapeutic agents that would allow double or triple therapies with lower systemic doses and significantly reduce undesirable side effects. Studies by Shams et al.[130] have proposed the principle for the use of TPDT as an adjuvant therapy for enhancement of antitumour immunity that may be capable of controlling distant disease through the active targeting of receptors that are overexpressed in CSCs. Currently, in spite of studies evaluating treatment methods targeting CCSCs, all strategies involving PDT treatment are under-test theories. Studies published so far are only beginning to investigate the ways to overcome CCSCs' resistance to PDT, and therefore, studies need to be considered evaluating combinative TPDT regimes that could effectively control CRC metastatic tumour growth and reoccurrence by eliminating CCSCs.

The evidence from this review has suggested that an active TPDT-enhanced PS drug delivery system might be a comprehensive strategy to improve CRC treatments, for example, designing an NP which is conjugated to three crucial elements: (1) a molecule for targeting specific CCSC, (2) a PS drug to eliminate CRC and (3) a chemosensitizer to overcome drug resistance. Such a combination would exert the anti-tumour TPDT effect with fewer side effects. However, the identification of strategies that exploit the unique characteristics of CSCs requires further study and the cooperation of multidisciplinary areas in order to enhance the overall PDT treatment modality for CCSCs.

Acknowledgements

The authors sincerely thank the University of Johannesburg, the National Laser Centre and the National Research Foundation – South African Research Chairs Initiative (NRF-SARChI) for their financial support through grant. The material in this research paper submitted to *Tumor Biology* has neither been published nor being considered elsewhere for publication.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work is based on the research supported by the South African Research Chairs Initiative of the Department of Science and Technology and National Research Foundation of South Africa (Grant No. 98337).

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