New strategies to enhance photodynamic therapy for solid tumors
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Chapter 1
Introduction, aims, and thesis outline

1. Photodynamic therapy

Cancer is one of the major causes of death in Europe with an incidence of 3.45 million cases/year and 1.75 million deaths in 2012. The disease arises as a result of pre-existing or acquired DNA mutations that ultimately cause genomic instability, uncontrolled cell division, tumor formation, organ dysfunction, which culminates in death when the disease cannot be managed. In modern medicine, tumors are predominantly treated with either surgery, chemotherapy, radiotherapy, or combinatorial treatment regimens. However, when these classical treatments fail or are associated with severe adverse events, patients rely on alternative interventions in order to achieve cure or disease management. Therefore, the development of new therapies and the improvement of existent treatments for cancer is a societal-, medical-, and economic necessity.

Photodynamic therapy (PDT) is a tumor-treatment that has been clinically approved for the treatment of a large variety of tumors. High complete tumor response rates (>70%) are achieved in the treatment of skin lesions, early central stage lung tumors, and esophageal malignancies, whereas relatively poor complete response rates (<50%) are obtained in the treatment of nasopharyngeal carcinomas and bladder tumors. Patients with extrahepatic cholangiocarcinoma (EHCC) are of special interest for treatment with PDT, since 70% of EHCC patients have non-resectable tumors and no curative treatment options are currently available. Palliative chemo- and radiotherapy for EHCC is relatively unsuccessful, whereas PDT has shown significant increases in the median survival time of these patients (from 6-9 months to ~14 months, respectively).

PDT entails the topical or systemic administration of a photosensitizer and the subsequent irradiation of the tumor with light that corresponds to the absorption peak of the photosensitizer, resulting in its excitation. The excited photosensitizer avidly reacts with oxygen to form reactive oxygen species (ROS). In turn, these ROS oxidize lipids, proteins, and other biological substrates, culminating in cell death. When administered systemically in free form, conventional photosensitizers localize in tumor vascular endothelium and tumor cells, and their demise following PDT results in thrombosis, blood flow stasis, and tumor tissue hypoxia that instills massive tumor cell death and attracts leukocytes that prompt a prolonged anti-tumor immune response.

However, compared to conventional PDT strategies, there are several factors that contribute to poor therapeutic efficacy in e.g., recurrent bladder tumors, nasopharyngeal carcinomas, and EHCCs. These culprits include suboptimal first- and second-generation photosensitizers, contemptible biodistributal properties, and a natural predisposition of tumor cells to survive PDT and adapt to post-therapeutic conditions.

2. Improving PDT for therapy-resistant tumors.

2.1 Selecting a more suitable photosensitizer

As described by Pleatzer et al., the ideal photosensitizer for PDT should absorb light in the therapeutic optic window between 650 and 850 nm. The first-generation photosensitizers hematoporphyrin derivative and its semi-purified form porfimer sodium absorb light at relatively low molar extinction coefficients (1 × 10³ M⁻¹ cm⁻¹) at 630 nm. Consequently, the excitation light is
poorly absorbed by the target chromophore and also absorbed and scattered by biological compounds such as melanin and hemoglobin derivatives, resulting in limited light penetration in tissue.

In order to improve PDT, there has been an ongoing search for photosensitizers with better optical properties. Many of the second-generation photosensitizers have absorption peaks at longer wavelengths, which permit deeper penetration of the excitation light into the tissue. Secondly, molar extinction coefficients are 10-100 times higher than first-generation photosensitizers. Thirdly, multiple intracellular and intratumoral loci are targeted, which benefits PDT efficacy, as elaborately reviewed by O'Connor et al. Combined, these factors increase the efficiency by which photosensitizers (especially those located deeper in the tumor tissue) are excited and thus contribute to more extensive ROS production necessary for successful tumor eradication.

Metallated phthalocyanines are second-generation photosensitizers that hold several important advantages over other first- and second generation photosensitizers. First of all, the absorption peak of phthalocyanines lies at 674 nm, well within the optic therapeutic window for photosensitizers. Secondly, its molar extinction coefficient is $2.74 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$, which is substantially higher than currently approved first- and second-generation photosensitizers, translating to much more efficient photon absorption. Thirdly, the central metal ion is interchangeable, allowing the tuning of physicochemical properties of the compound such as triplet state lifetime and singlet oxygen quantum yield. Fourth, metallated phthalocyanines do not exert any toxicity in the absence of light (dark toxicity), in sheer contrast to more conventional photosensitizers. Metallated phthalocyanines are readily available in hydrophobic and hydrophilic variants, of which the non-modified zinc phthalocyanine (ZnPC) is most abundantly studied.

**2.2 Targeted delivery of the photosensitizer towards tumor tissue.**

Systemically administered PSs are known to accumulate in non-tumor tissue, of which the exposure to light causes significant adverse events (skin toxicity). Due to prolonged skin photosensitivity (non-specific biodistribution of the photosensitizer), PDT has been discontinued by many medical centers, including the Academic Medical Center of the University of Amsterdam, for the treatment of these patients. Therefore, improving the efficacy of PDT and reducing non-specific cutaneous accumulation with targeted PSs may be of great benefit for cancer patients.

As a result of non-specific uptake of anticancer drugs and associated adverse events, a plethora of chemotherapeutic agents have been encapsulated into liposomes in order to ameliorate these drawbacks. Liposomes are biologically-compatible vesicles composed of a lipid bilayer and an aqueous core that are capable of carrying lipophilic and hydrophilic drugs. The lipid bilayer can be chemically modified or functionalized to bestow specific biodistributive properties upon the liposomes. Examples of biochemical modifications include the coating of the liposomes in a steric barrier of poly-ethylene glycol to give it stealth-like properties, the inclusion of electropositive or electronegative charges, and the bioconjugation of antibodies onto the liposomes that recognize e.g., tumor-specific antigens. Besides biochemical properties, the liposomal size can easily be modified, resulting in an additional biopharmaceutical variable that can be optimized to achieve the desired biodistributive properties.

**2.3 Prevention of tumor cell survival following PDT**

Although the physicochemical properties and biodistributive restrictions of photosensitizers are factors that can be optimized for current PDT regimens, a third factor that is highly important for the outcome of PDT is related to tumor biology. PDT induces oxidative stress and causes vascular shutdown that result in hypoxia and hyponutrition of the tumor tissue. Given that oxidative stress and hypoxia are often preexisting conditions within the tumor tissue, and tumor cells may therefore be primed to cope with the destructive effects of PDT. Moreover, since hallmarks of cancer include genetic instability and unlimited and unrestrained proliferation leading to variations in tumor cell
genotype and phenotype, PDT induces a stressor that naturally selects the tumor cells best capable of adapting to oxidative stress, hypoxia, and hyponutrition. Thus, there is a clear need to identify the survival mechanisms that tumor cells engage to cope with PDT-induced oxidative damage, hypoxia, and hyponutrition as these may reduce tumor response to therapy and promote disease recurrence. It is hypothesized that the identification of these survival mechanisms and their subsequent pharmacological inhibition will exert an adjuvant effect on PDT efficacy.

3. Aim of thesis

The aim of this thesis was to devise new strategies to improve PDT for solid tumors. It was hypothesized that the efficacy of PDT could be substantially improved by (1) utilizing ZnPC as a photosensitizer, (2) to target ZnPC to three pharmacologically relevant sites within the tumor using liposomal drug delivery, (3) to pharmacologically modulate tumor cell adaptation mechanisms, and (4) to activate auxiliary cell death pathways during or following PDT.

4. Thesis outline

The thesis initially focuses on the development and testing of liposomal formulations that contain ZnPC as a photosensitizer. A theoretical rationale for the use of ZnPC in combination with liposomal drug delivery is given in Chapter 2. Chapter 3 is a biochemical engineering approach to the development and testing of “interstitially-targeted liposomes” (ITLs) that are intended to passively yet specifically target to the tumor stroma. Similarly, Chapter 4 describes the preparation of “tumor cell-targeted liposomes” (TTLs), i.e., liposomes that are functionalized with tumor cell-recognizing antibodies that allow the specific delivery of ZnPC to the tumor cells. Chapter 5 is an elaborate in vitro and in vivo characterization of ZnPC-containing liposomes that are taken up specifically by tumor vascular endothelium, which were termed “tumor vascular endothelium-targeted liposomes” (ETLs).

Secondly, this thesis describes the investigation of adjuvant agents that can be co-encapsulated into the abovementioned liposomal formulations. Chapter 6 is a molecular and cellular biology perspective on the adaptive pathways that tumor cells initiate in order to survive PDT and identifies pharmacological agents that can be utilized to enhance PDT efficacy. Chapter 7 is a transcriptomics study that confirms the activation of these pathways in human cholangiocarcinoma cells. Pharmacological inhibition of one of these pathways, namely the hypoxic survival pathway, is brought into practice in Chapter 8 and Chapter 9, in which the neoadjuvant use of acriflavin was used in combination with PDT to increase its therapeutic efficacy by reducing cell survival under hypoxic post-therapeutic conditions. The inhibition of the inflammatory response mediated by the nuclear factor kappa B transcription factor is explored in Chapter 10, with special emphasis on the effects on the anti-tumor immune response. Chapter 11 explores the feasibility of adjuvant tirapazamine to activate hypoxia-inducible DNA damage in combination with PDT to activate auxiliary cell death pathways not typically induced by conventional PDT.

References