Chapter 22

Theranostic Near-Infrared Photoimmunotherapy

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22.1 Introduction

Targeted cancer therapies offer the promise of highly effective tumor control with fewer side-effects than conventional cancer treatments. In this approach, drugs or radioisotopes are directed to a tumor by coupling to monoclonal antibodies (mAbs) against specific targets on the cancer cell surface. These antibody–drug conjugates (ADCs) have had modest commercial success, but side-effects remain problematic. We have greatly advanced targeted cancer detection that assists surgical or endoscopic therapy by developing a series of optical imaging probes (‘activatable probes’) that only fluoresce when they are bound to or inside tumors (Kobayashi et al. 2010; Kobayashi and Choyke 2011), enabling precise tracking of cancer cells and drugs in the tissue (Urano et al. 2009). With these probes, cancer-specific fluorescence has been achieved in animal models and in fresh surgical specimens from cancer patients.

From a physics perspective, excitation energy can be dissipated in the form of fluorescent light for diagnosis. Alternatively, it can be exchanged for heat, oxidation or photochemical reactions to induce cytotoxic treatment. Our work is to be differentiated from conventional photodynamic therapy (PDT). PDT has been in limited clinical use for several decades. Photofrin (porfimer) is the most commonly employed PDT agent and has been used to treat endobronchial, esophageal and bladder cancers. Photofrin and related compounds passively permeate into cells based on their

Electronic Supplementary Material The online version of this chapter (https://doi.org/10.1007/978-981-13-7908-6_22) contains supplementary material, which is available to authorized users.

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hydrophobicity which is non-specific but slightly favors uptake in cancer cells and when exposed to light in the presence of oxygen, generate reactive oxygen species. Reactive oxygen species are typically toxic to the cell, resulting in cell death, however, this typically occurs within the cytoplasm and results in apoptosis rather than necrosis. Although PDT is used in specific cases, widespread adoption has been limited because the current compounds distribute rather non-specifically and permeate into normal cells leading to off-target toxicities. For instance, patients are often unable to go outside into direct sunlight for several months and even room light for several days. Incidental exposure to strong light, for instance, from a copy machine, can be catastrophic. Moreover, PDT is notorious for inducing serious inflammatory changes at the treatment site, limiting its utility. This relates to the non-specific bio-distribution and slow clearance rates (half-life of 23 days) of PDT agents. More general use of PDT is thus limited by its potential side effects. Targeted PDT has been attempted but delivery issues related to the large number of photosensitizer molecules in each conjugate, limits the achievable concentrations of the photosensitizer.

By extending the target-cell specific fluorescence imaging methodology from ‘see’ for cancer detection to ‘kill’ for cancer therapy, we then developed a new form of ADC comprised of an mAb attached to a photoabsorbing phthalocyanine-based chemical, termed IRDye700DX (IR700). When this conjugate is injected and the target cancer tissue is illuminated with harmless near-infrared light of wavelength 690 nm, the IR700 part of the molecule becomes activated and splits, turning hydrophobic, which compromises the cell membrane, thereby killing the cancer cell. This approach is safer than other conventional ADCs because it only kills illuminated cells that bind mAb–IR700 conjugates. Since 690 nm light penetrates skin and tissue to several centimeters in depth without damaging any normal cells, the therapy can access most organs from the surface or via endoscopy or fine optical fiber insertion through a transparent catheter needle without surgery. Moreover, the loss of fluorescence upon activation allows therapeutic effects to be monitored in real time. We termed this new form of phototherapy ‘near-infrared photoimmunotherapy’ (NIR-PIT) (Mitsunaga et al. 2011) (Fig. 22.1).

22.2 NIR-PIT Can Selectively Kill Various Cancer Cells

We have shown that this approach works for numerous molecular targets and cancer types. By simply changing the antibody, NIR-PIT can target a broad array of cancer-specific target molecules including the proteins EGFR, HER2, PSMA, CD25, CEA, Mesothelin, GPC3, CD20 and PD-L1, among others. When NIR-PIT was employed for targeting cancer cells to be killed in animal models, we observed significant tumor shrinkage after a single administration of the conjugate and NIR light, and repeated exposure to NIR light produced a more than 80% reduction of the exposed tumors with prolonged disease-free survival and without evident adverse side-effects. Since NIR-PIT can achieve spatially selective killing of target cells, it can be used to eliminate cells containing cancer stem cell markers such as CD44 (Jin et al. 2016) and CD133 (Jing et al. 2016) as we have demonstrated for

Near infrared photoimmunotherapy (NIR-PIT)

Fig. 22.1 Scheme explaining the basis of near-infrared photoimmunotherapy (NIR-PIT)

breast cancer and glioblastoma stem cells, respectively, without harming normal stem cells expressing these markers in other parts of the body. Targeting cancer stem cells in this way suppresses tumor regrowth for long periods (Fig. 22.2).

22.3 NIR-PIT Rapidly Enhances Nano-Drug Delivery

In addition, NIR-PIT has a desirable side-effect: it initially causes enlargement of the tumor vasculature, increasing blood flow and permeability that induces enhanced delivery of various size of nano-drugs ranging from 5 to 300 nm in diameter.

To date, the delivery of nano-sized therapeutic agents to cancers has been disappointing based as it is on enhanced permeability and retention (EPR) caused by the relatively leaky nature of cancer vasculature. As a result of EPR, there is a modest delivery of nano-sized agents which, although promising as a class of drugs, have demonstrated limited success in oncology. For instance, the EPR effect is estimated to increase nano-drug delivery 20–30% in most cases and 200% in the best case scenario compared with normal tissues. However, after NIR-PIT we observed dramatically increased permeability and retention in the treated tumor bed. We termed this phenomenon, super-enhanced permeability and retention effects (SUPR). Post NIR-PIT SUPR dramatically enhances the delivery of nano-sized agents into treated tissue by up to 24-fold compared with conventional EPR effects (Sano et al. 2013). SUPR occurs because the initial NIR-PIT only kills antigen expressing tumor cells in the perivascular space while leaving the tumor vessels intact, enabling a striking increase in blood volume and, therefore, nano-drug perfusion into the tumor bed

Fig. 22.2 Scheme explaining the mechanism of near-infrared photoimmunotherapy (NIR-PIT) induced super-enhanced permeability and retention (SUPR) effects

within tumor vessels. This permits the delivery of dramatically higher concentrations of nano-sized drugs into treated tumors with minimal uptake in untreated regions. Thus, after NIR-PIT the delivery of nano-drugs into tumors results in highly efficient tumor killing of cells surviving NIR-PIT with minimal side effects to other organs as therapeutic effects can be achieved at much lower doses (Fig. 22.3).

22.4 NIR-PIT Initiates Anti-Tumor Host Immunity and Promotes Rapid Healing

Three-dimensional dynamic quantitative phase-contrast microscopy (QPM) and dual selective plane illumination microscopy (dSPIM) of tumor cells undergoing PIT showed rapid swelling in treated cells immediately after light exposure suggesting rapid water influx into cells, followed by irreversible morphologic changes such as bleb formation, and rupture of cellular membrane and vesicles. Furthermore, biological markers of ICD including relocation of HSP70/90 and calreticulin from cytosol to cellular membrane, and release of calreticulin, ATP and High Mobility Group Box 1 (HMGB1), were clearly detected immediately after NIR-PIT. When NIR-PIT was performed in a mixed culture of cancer cells and immature dendritic

Fig. 22.3 Diagram of the applications of NIR-PIT

cells, maturation of immature dendritic cells was strongly induced rapidly after NIR-PIT against cancer cells.

Because cell membranes across mammalian species exhibit virtually identical physico-chemical properties, they are equally susceptible to the photochemical damage induced by NIR-PIT. Thus, new NIR-PIT conjugates can be developed *in vitro*, *ex vivo* or in animal models with a very high likelihood of successful translation to human patients. This translatability is an important advantage of our chemistry- and photophysics-based approach to cancer treatment. NIR-PIT technology opens the doors for many clinical applications and we hope it will lead to new treatments for numerous different cancer types. In addition, we have found that intact tissue stem cells in the tumor bed greatly contribute to clean wound healing, vital for improving the prognosis and quality of life of cancer patients treated with NIR-PIT.

22.5 Targeting Systemic Metastases

Furthermore, NIR-PIT also shows great promise as an indirect cancer immunotherapy. NIR-PIT achieved spatially selective depletion of tumor-associated immunosuppressing regulatory T cells (Tregs), which inhibit anti-tumor attack (and autoimmunity) by cytotoxic CD8+T cells that proliferate within a tumor. Eliminating Tregs locally in a tumor bed allows the adjacent cytotoxic CD8+T and natural killer (NK) cells to instantly attack the tumor within 1 h. Remarkably, Treg-targeting NIR-PIT also caused the selective systemic regression of untreated distant metastatic tumors with the same cell origin as the treated tumor within 2 days, presumably because once awakened, cytotoxic CD8+T cells were no longer susceptible to

Mechanism of Treg-targeting NIR-PIT

Fig. 22.4 Scheme explaining the functional mechanism of Treg-targeting near-infrared photoimmunotherapy (NIR-PIT)

Treg-induced inactivity (Sato et al. 2016). In contrast, awakened cytotoxic CD8+T cells did not attack normal cells or other cancer cells, ensuring that Treg-targeting NIR-PIT was a highly cell-selective cancer therapy with minimal autoimmune adverse side-effects of the type seen with systemic cancer immunotherapies that activate host-immunity throughout the body (Fig. 22.4).

22.6 Perspective

NIR-PIT shows immense promise for practical and clinical applications. Several NIR-PIT-related patents were licensed to the start-up biotech company Aspyrian Therapeutic Inc., which started a phase I clinical trial in June 2015 and currently advanced to phase II, using the cetuximab-IR700 conjugate (RM-1929) to treat head and neck cancer patients who had failed to respond to all conventional cancer therapies including surgery, chemotherapy and radiation therapy (<https://clinicaltrials.gov/ct2/show/NCT02422979>). Similar trials are planned for lung, esophageal, bladder and pancreatic cancer, some precancerous conditions including leukoplakia and papillomatosis, and others by targeting cancer cells or immune-suppressor cells in the near future. We have engaged researchers internationally to further explore the possibilities of NIR-PIT and to expedite its introduction into the clinic.

Acknowledgements This research was supported by the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Center for Cancer Research.

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