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Light-controlled endosomal escape of the novel CD133-targeting immunotoxin AC133–saporin by photochemical internalization — A minimally invasive cancer stem cell-targeting strategy

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ABSTRACT

The cancer stem cell (CSC) marker CD133 is an attractive target to improve antitumor therapy. We have used photochemical internalization (PCI) for the endosomal escape of the novel CD133-targeting immunotoxin AC133–saporin ($PCI_{AC133-saporin}$). PCI employs an endocytic vesicle-localizing photosensitizer, which generates reactive oxygen species upon light-activation causing a rupture of the vesicle membranes and endosomal escape of entrapped drugs. Here we show that AC133–saporin co-localizes with the PCI-photosensitizer $TPCS_{2a}$, which upon light exposure induces cytosolic release of AC133–saporin. PCI of picomolar levels of AC133–saporin in colorectal adenocarcinoma WiDr cells blocked cell proliferation and induced 100% inhibition of cell viability and colony forming ability at the highest light doses, whereas no cytotoxicity was obtained in the absence of light. Efficient PCI-based CD133-targeting was in addition demonstrated in the stem-cell-like, triple negative breast cancer cell line MDA-MB-231 and in the aggressive malignant melanoma cell line FEMX-1, whereas no enhanced targeting was obtained in the CD133-negative breast cancer cell line MCF-7. $PCI_{AC133-saporin}$ induced mainly necrosis and a minimal apoptotic response based on assessing cleavage of caspase-3 and PARP, and the TUNEL assay. $PCI_{AC133-saporin}$ resulted in S phase arrest and reduced LC3-II conversion compared to control treatments. Notably, co-treatment with Bafilomycin A1 and $PCI_{AC133-saporin}$ blocked LC3-II conversion, indicating a termination of the autophagic flux in WiDr cells. For the first time, we demonstrate laser-controlled targeting of CD133 in vivo. After only one systemic injection of AC133–saporin and $TPCS_{2a}$, a strong anti-tumor response was observed after $PCI_{AC133-saporin}$. The present PCI-based endosomal escape technology represents a minimally invasive strategy for spatio-temporal, light-controlled targeting of CD133 + cells in localized primary tumors or metastasis.

1. Introduction

Aggressive cancer cells with normal stem-like properties are termed cancer stem cells (CSCs) or tumor initiating cells (TICs), and are proposed to drive tumor progression, therapy resistance and metastasis [1]. Monoclonal antibodies (mAbs) targeting human CD133 has been used to identify and isolate CSCs from different cancers including colon carcinoma [2–4], breast carcinoma [5,6], malignant melanoma [7], pancreatic carcinoma [8], glioma [9,10] and ovarian cancer [11,12]. $CD133^{high}$ cells are able to self-renew by symmetric or asymmetric cell division thereby maintaining both CSC properties as well as tumor heterogeneity upon serial transplantations in vivo [2,3]. Of clinical relevance, overexpression of CD133 has been correlated with clinical outcome and significantly linked to malignant transformation or poor clinicopathological parameters in colorectal cancer [13–15] and has been associated with recurrence in breast cancer patients [16]. Gamma-radiation of CD133 + glioma stem cells induces autophagy which contributes to radioresistance [17]. In addition, ALA-PpIX-mediated photodynamic therapy (PDT) can also induce autophagy that promotes resistance in CD133 + colon cancer stem-like cells [18]. Normal stem cells express CD133 [19], and hence it is of high importance that CD133-targeting anti-cancer stem cell (CSC) strategies provide a specific and efficient delivery of cytotoxic drugs only in the $CD133^{+}$ cells of the tumor.

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Photochemical internalization (PCI) is a laser-controlled efficient and specific intracellular drug delivery technology suggested to be an attractive strategy for the selective ablation of CSC populations in primary tumors or localized metastases [20–24]. PCI is based on the use of an amphiphilic photosensitizer (e.g. $TPCS_{2a}$/Amphinex) that together with hydrophilic anti-cancer drug(s), e.g. toxin payloads, co-localize in endocytic vesicles where $TPSC_{2a}$ is inserted in the membrane of endosomes and the hydrophilic drug is localized in the lumen of the same vesicles. In the presence of molecular oxygen, visible light activation (652 nm laser light) of $TPCS_{2a}$ induces the generation of reactive oxygen species (ROS) causing lipid peroxidation and subsequently rupture of the endolysosomal membranes and release of the sequestered drug of interest into the cytosol [25,26]. The PCI method is presently under evaluation in two clinical trials; one Phase II trial in combination with bleomycin for the treatment of squamous cell carcinoma of the head and neck [27] and one Phase I trial in combination with gemcitabine for the treatment of inoperable bile duct cancer [28]. PCI has also been proven highly effective for improving the cytosolic delivery of toxins and targeted toxins based on type I ribosome inactivating protein (RIP) toxins such as saporin and gelonin in pre-clinical studies [20,24,29–34]. Type I RIPs are lacking the ability to escape from endolysosomal compartments and, hence, prone to degradation by lysosomal enzymes [35]. However, saporin (saporin-S6) is a highly potent toxin when released into the cytosol [36] and 1–10 RIP molecules are estimated sufficient to kill one cell [37]. Recently, specific and efficient $TPCS_{2a}$-PCI-based targeting of $CD133^{high}$ CSC-like cells from colon carcinoma and liposarcoma cells, by using a model immunotoxin complex consisting of the biotinylated mAb clone AC133 linked to streptavidin–saporin, was demonstrated [20,24]. The $CD133^{high}$ population, of the colon carcinoma cell line (WiDr), had a potent tumor-initiating capacity (10 cells per mouse) and was found to be PDT-resistant. However, PCI of AC133–saporin circumvented the PDT resistance and induced 100% killing of the WiDr cells down to subpicomolar levels [20]. Efficient PCI-based targeting of the putative CSC marker CD44 was also demonstrated using the same principle with biotinylated monoclonal antibody (IM7) targeting all variants of CD44 and bound to streptavidin–saporin (IM7-saporin) [21]. However, these CD133- and CD44-targeting model immunotoxin complexes are most truly too large (>700 kDa) for efficient penetration through solid tumor tissues and delivery into CSCs that are distant from the tumor vasculature.

The aim of the present study was to investigate the in vitro and in vivo potential of PCI of the novel 244 kDa version immunoconjugate AC133–saporin ($PCI_{AC133-saporin}$) for targeting CD133-expressing cancer cells. Targeting of CD133 + WiDr tumors with $PCI_{AC133-saporin}$ after only one systemic injection and one light exposure gave a significant delay in tumor growth compared to sub-lethal doses of $PCI_{No\ drug}$ ($TPCS_{2a}$ + light) or AC133–saporin alone, suggesting this PCI-based approach as a rational minimal-invasive strategy for the elimination of CD133-expressing CSCs.

2. Materials and methods

2.1. Cell lines and routine culturing

The human colorectal adenocarcinoma cell line WiDr ($CD133^{high}$), the breast cancer cell lines MDA-MB-231 ($CD133^{+}$) and MCF-7 ($CD133^{-}$ control cells) and the acute lymphocytic leukemia cell line Reh (used as control cells for assessment of apoptotic response), were obtained from ATCC (Rockville, MD, USA) and cultured under routine conditions in standard RPMI medium (Sigma Aldrich, MO, USA) or Minimum Essential Medium Eagle (MCF-7 cells) (Sigma Aldrich) supplemented with 10% fetal bovine serum (FBS) (PAA Laboratories GmbH, Pasching, Austria), 100 U/ml penicillin and 100 μg/ml streptomycin (Sigma Aldrich). The human malignant melanoma cell line FMEX-1 ($CD133^{high}$) was a generous gift from the Department of Tumor Biology at the Norwegian Radium Hospital and was cultured in RPMI medium as described above. All the cell lines were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 and sub-cultured twice a week. The identity of WiDr, MDA-MB-231 and FMEX-1 cells was confirmed by short tandem repeat (STR) profiling using Powerplex 16 (Promega, Madison, WI, USA). The cell lines were mycoplasma negative during the study.

2.2. Chemicals

Meso-tetraphenyl chlorin disulfonate ($TPCS_{2a}$/Amphinex®, PCI Biotech AS, Lysaker, Norway) was used as the PCI-photosensitizer in this study. $TPCS_{2a}$ (in 3% polysorbate 80, 2.8% mannitol, 50 mM Tris pH 8.5, all from Sigma Aldrich, USA) stock solution (0.4 mg/ml) was kept at 4 °C in aliquots and light protected. All work with the $TPCS_{2a}$ was performed under subdued light.

2.3. Immunotoxin

The human CD133-targeting mAb AC133 was produced in the hybridoma cell line AC133.1 (ATCC # HB-12346), isolated and purified at Diatec Monoclonals, Oslo, Norway. The conjugation of the anti-human CD133 (clone AC133) mAb (Diatec Monoclonals) to saporin was custom conjugated by Advanced Targeting Systems (ATS), CA, USA, making the immunotoxin AC133–saporin (CS-BR, Lot #85-78). The conjugate was determined by ATS to be >90% pure and to have a concentration of 2.1 mg/mL and after standard chemical procedure to have an average ratio of 2.8 mol saporin/mol AC133 with an average molecular weight of 244 kDa. According to the manufacturer, CD133-saporin should be stable for up to 1 week at room temp, 1 month at 4 °C, and >1 year at −80 °C when stored undiluted. AC133–saporin and the toxin saporin (ATS) were stored in aliquots at −80 °C.

2.4. In vitro light source

Illumination of the cells and thereby excitation of $TPCS_{2a}$ was performed by using the Lumisource® lamp (PCI Biotech, Oslo) as previously described [20].

2.5. In vitro photochemical internalization of AC133–saporin ($PCI_{AC133-saporin}$)

Cells were seeded at 3×10^3 (WiDr), 12×10^3 (MDA-MB-231) 15×10^2 (FMEX-1) and 8×10^3 (MCF-7) cells/well in 96-well plates (Nunclon, Nunc, Roskilde, Denmark) and allowed to attach at 37 °C overnight. The cells were incubated for 18 h with 0.2 μg/ml $TPCS_{2a}$ ($PCI_{No\ drug}$) or, for the PCI experiments, co-incubated with 8.6 pM of AC133–saporin ($PCI_{AC133-saporin}$) or saporin ($PCI_{saporin}$) as a non-targeting control. As controls, we used untreated cells and cells incubated with only AC133–saporin (8.6 pM) or saporin (24 pM). The cells were subsequently washed twice with drug free culture medium before replacement with culture medium and chased for 4 h before they were exposed to increasing doses of light. Illuminations of the cells were performed by using Lumisource® (PCI Biotech AS, Lysaker) as described above.

2.6. Cytotoxicity assays

Cell viability after the different treatment regimes was assessed by using the MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyltetrazolium bromide) method 72 h after light exposure with Lumisource® as recently described [20]. The colony formation assay was performed to measure cell survival 10–14 days post treatments. WiDr cells (500 cells/well) were seeded out in 6 well plates (Nunc) and allowed to adhere overnight and subjected to the different treatments as described in Section 2.5 and processed as in [20]. Only colonies with ≥50 cells were judged as valid colonies and counted [38]. Real-time