MOLECULAR ONCOLOGY XXX (2016) 1–11

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Near infrared photoimmunotherapy of B-cell lymphoma

*Tadanobu Nagaya, Yuko Nakamura, Kazuhide Sato, Toshiko Harada, Peter L. Choyke, Hisataka Kobayashi**

Molecular Imaging Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892, United States

ARTICLE INFO

Article history:
Received 25 May 2016
Received in revised form
19 July 2016
Accepted 22 July 2016
Available online ■

Keywords:
Near infrared photoimmunotherapy
CD20
B-cell lymphoma
Monoclonal antibodies
Near infrared light
Molecular imaging

ABSTRACT

Near infrared photoimmunotherapy (NIR-PIT) is a new, highly-selective cancer theranostics that employs an antibody-photo absorber conjugate (APC). NIR-PIT has successfully treated preclinical tumor models with APCs and is now in the first-in-human phase 1 clinical trial for head and neck cancer patients against EGFR. CD20 is highly expressed in many B-cell lymphomas and is emerging as a molecular target for this disease. Here, we describe the use of the anti-CD20 monoclonal antibody (mAb), rituximab-IR700 APC for NIR-PIT of B-cell lymphoma in two CD20-expressing lymphoma mouse models. CD20 expressing B-cell lymphoma cell lines (Daudi and Ramos) were used in this study. Rituximab-IR700, rituximab conjugated with IRDye700DX, showed specific binding, and cell-specific killing only after exposure of NIR light to both cells *in vitro*. To evaluate effects of NIR-PIT *in vivo*, tumor-bearing mice were separated into 4 groups: (1) control; (2) APC i.v. only; (3) NIR light exposure only; (4) APC and NIR light (NIR-PIT). These were performed every week for up to 3 weeks. Rituximab-IR700 showed high tumor accumulation and high target-to-background ratio *in vivo*. Tumor growth was significantly inhibited by NIR-PIT in comparison with the other groups ($p < 0.001$ for both tumors), and survival was significantly prolonged in both tumors ($p < 0.001$ for Daudi tumors and $p < 0.0001$ for Ramos tumors vs other groups). More than half of tumors were cured with this single regimen of NIR-PIT. In conclusion, anti-CD20 rituximab-IR700 works as a highly effective APC for NIR-PIT against B-cell lymphoma.

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1. Introduction

CD20 is a B-cell antigen that is expressed from the late pre-B-cell stage until its loss just prior to terminal differentiation into plasma cells. CD20 is thought to have a regulatory function on B-cell proliferation and differentiation (Tedder and Engel, 1994). As a cell surface protein, CD20 is highly expressed in many B-cell lymphomas, so CD20 has become an important target for antibody-based therapies of various B-cell lymphomas. Rituximab as an anti-CD20 monoclonal antibody

Abbreviations: ADCC, antibody-dependent cell-mediated cytotoxicity; APC, antibody-photo absorber conjugate; CD, cluster of differentiation; CDC, complement-dependent cytotoxicity; DIC, differential interference contrast; EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; H&E, hematoxylin and eosin; IR700, IRDye700DX; LED, light-emitting diode; mAb, monoclonal antibody; NIR, near infrared; PI, propidium iodide; PBS, phosphate buffered saline; PIT, photoimmunotherapy; ROI, regions of interest; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TBR, target-to-background ratio.

* *Corresponding author*. Fax: +1 3014023191.
E-mail address: kobayash@mail.nih.gov (H. Kobayashi).

http://dx.doi.org/10.1016/j.molonc.2016.07.010
1574-7891/Published by Elsevier B.V. on behalf of Federation of European Biochemical Societies.

(mAb) is the first commercial antibody for treating malignancy which was approved by the Food and Drug Administration (FDA) (Leget and Czuczman, 1998). The anti-tumor activity of rituximab relies on antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) (Hamaguchi et al., 2006; Lefebvre et al., 2006; Reff et al., 1994; Uchida et al., 2004). Thus, rituximab, in combination with chemotherapy, has become an effective first-line or salvage therapy for B-cell lymphomas (Economopoulos et al., 2003; Griffin et al., 2009; Marcus et al., 2005; Wildes et al., 2014). However, such treatments are not curative in all patients, especially those with aggressive malignancies (Wasterlid et al., 2011, 2013).

Burkitt's lymphoma is a rare, highly aggressive type of mature B-cell neoplasm, accounting for approximately 1–2% of adult and 30–40% of childhood non-Hodgkin's lymphoma. Although much progress has been made, Burkitt's lymphoma remains a difficult disease to treat among B-cell lymphomas (Molyneux et al., 2012). For instance, conventional therapeutic regimens used in non-Hodgkin's lymphoma such as CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) have not been universally successful in patients with Burkitt's lymphoma (Wildes et al., 2014). Short, intensive regimens result in CR rates of 70–90%, and long-term event-free survival (EFS) rates between 45% and 90% (Wildes et al., 2014). However, this intensive multi-drug chemotherapy regimen also causes severe toxic side-effects including long lasting hematologic toxicity and mucositis that increase the risk of severe infection or chemotherapy-associated secondary neoplasm. Relapses after this and other intensive therapies are often lethal.

Near infrared photoimmunotherapy (NIR-PIT) is a tumor theranostics that employs a targeted monoclonal antibody-photo absorber conjugate (APC) including IRDye700DX (IR700, silica-phthalocyanine dye). NIR-PIT has been shown to be highly effective and selective (Mitsunaga et al., 2011). Unlike intensive chemotherapy, non-target expressing cells suffer no toxic effects after NIR-PIT. A first-in-human Phase 1 trial of NIR-PIT with an APC targeting epidermal growth factor receptor (EGFR) in patients with inoperable head and neck cancer was approved by the US FDA, and is underway (https://clinicaltrials.gov/ct2/show/NCT02422979).

NIR-PIT has been shown to be effective with a variety of different APCs however, anti-CD20, a commonly used antibody, has not been tested as an APC for NIR-PIT (Hanaoka et al., 2015; Mitsunaga et al., 2011; Nagaya et al., 2015, 2016b; Sato et al., 2015; Watanabe et al., 2015). In this study, we performed *in vitro* tumor binding, *in vivo* tumor accumulation and intratumoral distribution studies in animal models using two human aggressive B-cell (Burkitt's) lymphoma cell lines (Daudi and Ramos). Following this, NIR-PIT was performed with rituximab-IR700 *in vitro* and in two tumor bearing mouse models *in vivo* and efficacy was established.

2. Materials and methods

2.1. Reagents

Water soluble, silica-phthalocyanine derivative, IRDye700DX NHS ester was obtained from LI-COR Biosciences (Lincoln, NE, USA). Rituximab, a chimeric (mouse/human) monoclonal antibody (mAb) directed against CD20 was purchased from Genentech (South San Francisco, CA, USA). All other chemicals were of reagent grade.

2.2. Synthesis of IR700-conjugated rituximab

Conjugation of dyes with mAb was performed according to previous methods (Mitsunaga et al., 2011). In brief, rituximab (1.0 mg, 7 nmol) was incubated with IR700 NHS ester (61.1 μg, 31.3 nmol) in 0.1 M Na_2HPO_4 (pH 8.6) at room temperature for 1 h. The mixture was purified with a Sephadex G25 column (PD-10; GE Healthcare, Piscataway, NJ, USA). The protein concentration was determined with Coomassie Plus protein assay kit (Thermo Fisher Scientific Inc, Rockford, IL, USA) by measuring the absorption at 595 nm with UV–Vis (8453 Value System; Agilent Technologies, Santa Clara, CA, USA). The concentration of IR700 was measured by absorption at 689 nm to confirm the number of fluorophore molecules per mAb. The synthesis was controlled so that an average of two IR700 molecules was bound to a single antibody. We abbreviate IR700 conjugated to rituximab as rit-IR700. As a quality control for the conjugate, we performed sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The conjugate was separated by SDS-PAGE with a 4–20% gradient polyacrylamide gel (Life technologies, Gaithersburg, MD). A standard marker (Crystalgen Inc., Commack, NY) was used as a protein marker of molecular weight. After electrophoresis at 80 V for 2.5 h, the gel was imaged with a Pearl Imager (LI-COR Biosciences, Lincoln, Nebraska, USA) using a 700 nm fluorescence channel. We used diluted rituximab as a control. The gel was stained with Colloidal Blue staining to determine the molecular weight of the conjugate.

2.3. Cell culture

Epstein–Barr virus negative B-cell lymphoma cell lines, Daudi and Ramos, were purchased from American type culture collection (ATCC; Manassas, VA, USA). Cells were grown in RPMI 1640 (Life Technologies, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Life Technologies) in tissue culture flasks in a humidified incubator at 37 °C at an atmosphere of 95% air and 5% carbon dioxide.

2.4. Flow cytometry

To verify *in vitro* rit-IR700 binding, fluorescence from cells after incubation with the APC was measured using a flow cytometer (FACS Calibur, BD BioSciences, San Jose, CA, USA) and CellQuest software (BD BioSciences). Daudi and Ramos cells (4×10^5) were seeded into 12 well plates and incubated for 24 h. Rit-IR700 was then added to the culture medium at 10 μg/ml and incubated for 6 h at 37 °C. To validate the specific binding of the conjugated antibody, excess antibody (100 μg) was used to block 10 μg of APCs.

Please cite this article in press as: Nagaya, T., et al., Near infrared photoimmunotherapy of B-cell lymphoma, Molecular Oncology (2016), http://dx.doi.org/10.1016/j.molonc.2016.07.010