Analytical Biochemistry 504 (2016) 50–58

Contents lists available at ScienceDirect

Analytical Biochemistry

journal homepage: www.elsevier.com/locate/yabio

Rapid assessment of drug resistance of cancer cells to gefitinib and carboplatin using optical imaging

Zhen Luo [a], Kiana Michelle Samadzadeh [a], Nitin Nitin [a, b, *]

[a] Department of Biological and Agricultural Engineering, University of California, Davis, Davis, CA 95616, USA
[b] Food Science and Technology, University of California, Davis, Davis, CA 95616, USA

ARTICLE INFO

Article history:
Received 16 August 2015
Received in revised form
14 March 2016
Accepted 28 March 2016
Available online 5 April 2016

Keywords:
Molecular imaging
Molecular targeted therapy
Chemotherapy
Drug response
Metabolic activity

ABSTRACT

The overall goal of this study was to evaluate optical molecular imaging approaches to determine the drug response of chemotherapy and molecular targeted agents in drug sensitive and drug resistant cell lines. The optical molecular imaging approaches selected in this study were based on changes in intracellular uptake and retention of choline and glucose molecules. The breast cancer cell lines were treated with a molecular targeted anti-EGFR therapy. The bladder cancer cell lines were treated with a conventional chemotherapy approach. Sensitivity of optical molecular imaging approach was also compared with conventional cell viability and cell growth inhibition assays. Results demonstrate that optical molecular imaging of changes in intracellular uptake of metabolites was effective in detecting drug susceptibility for both molecular targeted therapy in breast cancer cells and chemotherapy in bladder cancer cells. Between the selected metabolites for optical molecular imaging, changes in glucose metabolic activity showed higher sensitivity in discrimination between the drug sensitive and drug resistant cell lines. The results demonstrated that optical molecular imaging approaches more significantly sensitive as compared to the conventional cell viability and growth assays. Overall, the results demonstrate potential of optical molecular imaging of metabolic activity to improve sensitivity of in-vitro drug response assays.

Current cancer treatments are often selected based on the prior experience of clinicians and recommendations from alliances such as the National Comprehensive Cancer Network. Therapy selection processes often do not involve analytical methods to determine the optimal treatment for individual patients. To personalize treatment for individual patients, significant research efforts are being made in complementary directions to predict the therapeutic response of individual patients without exposing them to toxic drugs. These approaches include genetic testing to identify patients who may benefit for chemo and/or hormone therapies [1] and ex vivo assessment of chemo sensitivity of tumor cells [2–8].

The clinical impact of selecting breast cancer patients for chemo or hormone therapy using genetic testing is well illustrated by the success of the Oncotype DX test among early stage ER + breast cancer patients [9]. This test, based on the analyses of 21 genes in an isolated primary tumor, assesses the risk of reoccurrence and predicts the potential benefit of chemo and hormone therapies. Despite significant potential, the current genetic testing including protein based molecular biomarkers cannot identify the optimal therapeutic regimen for a broad class of breast cancer patients (i.e., the particular combination of chemo and hormone therapy drugs) for individual patients or identify drug resistance in patients [10]. Furthermore, such genetic tests are still not available for cancers other than breast cancer, although progress is being made in developing such tests.

Complementary to the genetic approaches, ex vivo or in vitro chemo sensitivity assays such as histoculture drug response assays (HDRAs) can directly measure susceptibility of isolated tissues and cells to chemo therapies [11,12]. These HDRAs are aimed at

Abbreviations: HDRA, histoculture drug response assay; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; EGFR, epidermal growth factor receptor; PET, positron emission tomography; MR, magnetic resonance; 2-NBDG, 2-[*N*-(7-nitrobenz-2-oxa-1,3-diaxol-4-yl)amino]-2-deoxyglucose; ATCC, American Type Culture Collection; FBS, fetal bovine serum; DMEM, Dulbecco's modified Eagle's medium; PBS, phosphate-buffered saline; MFI, mean fluorescence intensity; FOV, field of view.

* Corresponding author. Department of Biological and Agricultural Engineering, University of California, Davis, Davis, CA 95616, USA.
E-mail address: nnitin@ucdavis.edu (N. Nitin).

http://dx.doi.org/10.1016/j.ab.2016.03.021
0003-2697/

measuring changes in cell viability in a minced tissue section (taken from a large biopsy) on exposure to the selected chemotherapy drug. These minced biopsy sections are embedded in a collagen gel and cultured in the presence of drug molecules for an extended period of time (4 or 5 days to 2 weeks). Following incubation, changes in cell viability of the minced tissue sections are measured using conventional assays such as the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. In this assay, a few grams of the tissue biopsy sample are often required for testing [13].

Few clinical studies have highlighted the success of using HDRAs to predict drug susceptibility in clinically isolated specimens [11,14,15]. Most of the patients evaluated in these clinical studies had an advanced stage of disease that was clinically found to be resistant to a standard treatment regimen. But despite significant potential of ex vivo chemo sensitivity assays in identifying drug susceptibility and resistance, these approaches are not widely used in a clinical setting because the current methods for chemo sensitivity assessment have significant limitations [16], including (i) variability in cell survival during extended culturing (~1 week) of samples isolated from patients, (ii) culture-induced changes in cell physiology that may influence the drug response, (iii) delay in treating patients due to an extended culturing of samples, (iv) lack of sensitivity to measure the response of individual cells in a heterogeneous disease such as cancer, and (v) requirement of a relatively large tissue biopsy. Furthermore, these conventional ex vivo chemo sensitivity assays are not effective for evaluating efficacy of molecular targeted therapy because these therapies do not induce rapid cell death, unlike the response in cells to conventional chemotherapy [17,18].

To address the current limitations of in vitro drug response assay, this study was aimed at evaluating optical molecular imaging approaches to determine the drug response of chemotherapy and molecular targeted agents in drug sensitive and drug resistant cell lines. The optical molecular imaging approaches are based on measuring changes in metabolic activity of cancer cells on treatment with chemo or molecular targeted therapy. This approach is motivated based on the clinical evidence that tumor tissues have elevated metabolic activity and that changes in metabolic activity induced by many chemotherapy drugs (e.g., cisplatin, paclitaxel) [19,20] or certain molecular targeted therapies (e.g., EGFR [epidermal growth factor receptor] inhibitors) can predict the response of tumor to treatment in vivo [21,22]. Based on these facts, both PET (positron emission tomography) imaging (probes such as ^{18}FDG and ^{11}C-choline) and MR (magnetic resonance) spectroscopy methods (for quantifying choline content) have been developed to measure changes in metabolic activity of tumor tissue. Although PET and MR imaging methods are useful for in vivo imaging of tumors, these methods have limited applications for ex vivo and in vitro drug response assays.

Optical molecular imaging is an alternative technology that has a potential for measuring changes in metabolic activity of individual cancer cells using optically detectable metabolic tracer probes. Drug response assessment in cancer cells using optical imaging methods has a potential to identify heterogeneity in drug responses and detect small portions of drug resistant cells. In this study, the overall goals were to evaluate the potential of optical molecular imaging approaches to measure the response of cancer cells to the selected molecular targeted and conventional chemo therapies and to differentiate between the drug resistant and sensitive cell lines for the selected therapies. In this study, cell lines from breast and bladder cancer were selected as model systems to demonstrate potential of the molecular imaging approach to detect drug response in diverse cancer types. For the bladder and the breast cancer model systems, a drug sensitive cell line and a drug resistant cell line were selected. A molecular targeted EGFR inhibitor therapy was selected for the breast cancer cell lines. Carboplatin, a chemotherapy drug, was selected for the bladder cancer cell lines. The drug response was measured based on changes in the intracellular uptake and retention of optical analogues of glucose and choline. The results of optical imaging measurements were compared with those of conventional cell viability assays. Overall, the results of this study demonstrate a novel approach to evaluate drug response of cancer cells to molecular targeted and chemo therapies. Successful development of this novel approach can address the limitations of the current HDRA measurements for chemotherapy drugs and extend the application of the current HRDAs to molecular targeted therapies. This novel approach has a significant potential to impact selection of personalized therapies for cancer patients.

Materials and methods

Chemicals

A 10-mM stock solution of carboplatin (Sigma–Aldrich, St. Louis, MO, USA) was prepared in deionized water. Gefitinib (ZD1839) was purchased from Selleck Chemicals (Houston, TX, USA). 2-[*N*-(7-nitrobenz-2-oxa-1,3-diaxol-4-yl)amino]-2-deoxyglucose (2-NBDG) was purchased from Invitrogen (Carlsbad, CA, USA). A 5-mg/ml stock solution of 2-NBDG was prepared in distilled water. The MTT cell proliferation assay kit was purchased from ATCC (American Type Culture Collection, Rockville, MD, USA). Propargyl choline was synthesized using a method described by Jao and coworkers [23]. Here, 4 g of propargyl bromide (80% solution in toluene) was added to 3 g of dimethyl ethanolamine in 10 ml of tetrahydrofuran. The mixture was stirred over ice for approximately 30 min. The reaction mixture was then stirred for 24 h under nitrogen. Propargyl choline (white solid) was separated from the solvent by filtration (Millipore filter), followed by repeated washing with tetrahydrofuran. The dried product was stored at −20 °C.

Cell culture

In this study, two breast cancer cell lines (BT-474 and MDA-MB-231) and two bladder cancer cell lines (5637–sensitive and 5637–resistant) were used. The BT-474 and MDA-MB-231 cell lines were purchased from ATCC. The BT-474 cell line was maintained in a culture medium consisting of RPMI 1640 (Fisher Scientific, Pittsburgh, PA, USA) supplemented with 10% fetal bovine serum (FBS; Fisher Scientific) and 100 mg/L penicillin (Sigma–Aldrich). The MDA-MB-231 cell line was maintained in a culture medium consisting of Dulbecco's modified Eagle's medium (DMEM; Fisher Scientific) supplemented with 10% FBS and 100 mg/L penicillin. Bladder cancer cell lines (5637–sensitive and 5637–resistant) were kindly provided by Chong-Xian Pan (University of California, Davis). These two bladder cancer cell lines were grown in RPMI 1640 (Invitrogen) supplemented with 10% FBS (Fisher Scientific) and 100 mg/L penicillin (Sigma–Aldrich). All cells were grown in a humidified atmosphere of 5% CO_2–95% air at 37 °C and subcultured with 0.05% trypsin (Invitrogen). For imaging of cells, cells were cultured on 8-well coverslip bottom culture chambers (Fisher Scientific).

Glucose uptake assay

Cancer cells grown on 8-well coverslip bottom culture chambers were treated with the selected drug molecules in the concentration range of 0–50 μM for 24 h. After drug treatment, supernatant was discarded and the cells were washed three times with phosphate-buffered saline (PBS). PBS-washed cells were then incubated in