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Following anticancer drug activity in cell lysates with DNA devices

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ABSTRACT

There is a great need to track the selectivity of anticancer drug activity and to understand the mechanisms of associated biological activity. Here we focus our studies on the specific NQO1 bioactivatable drug, ß-lapachone, which is in several Phase I clinical trials to treat human non-small cell lung, pancreatic and breast cancers. Multi-electrode chips with electrochemically-active DNA monolayers are used to track anticancer drug activity in cellular lysates and correlate cell death activity with DNA damage. Cells were prepared from the triple-negative breast cancer (TNBC) cell line, MDA-MB-231 (231) to be proficient or deficient in expression of the NAD(P)H:quinone oxidoreductase 1 (NQO1) enzyme, which is overexpressed in most solid cancers and lacking in control healthy cells. Cells were lysed and added to chips, and the impact of β-lapachone (β-lap), an NQO1-dependent DNA-damaging drug, was tracked with DNA electrochemical signal changes arising from drug-induced DNA damage. Electrochemical DNA devices showed a 3.7-fold difference in the electrochemical responses in NQO1+ over NQO1− cell lysates, as well as 10–20-fold selectivity to catalase and dicoumarol controls that deactivate DNA damaging pathways. Concentration-dependence studies revealed that 1.4 μM β-lap correlated with the onset of cell death from viability assays and the midpoint of DNA damage on the chip, and 2.5 μM β-lap correlated with the midpoint of cell death and the saturation of DNA damage on the chip. Results indicate that these devices could inform therapeutic decisions for cancer treatment.

1. Introduction

There is a great need to not only detect cancer at its onset, but also determine which cancer therapy balances drug effectiveness with minimal side-effects for a particular patient. For these reasons, it would be highly beneficial to develop sensors that can follow drug activity for cancer-selective treatments that can be applied to discover patient-specific responses (Patolsky et al., 2006). In addition, there is a great benefit to produce platforms that can quickly track the underlying biological activity of drugs in order to understand mechanisms of action, particularly with respect to DNA damage (Carozzi et al., 2015; Roos et al., 2016). Such platforms with modular control of intracellular components would enable the systematic investigation of biological pathways of drug activity for further understanding and refinement of targeted cancer lethality.

A number of cancer treatments cause DNA damage to bring about cell death, such as cisplatin, doxorubicin, and methotrexate (Cheung-Ong et al., 2013). Others are under development for selective targeting of cancer. Among these, we are investigating a drug, ß-lapachone (ß-lap, ARQ761 in clinical form), due to its great potential to treat a number of aggressive solid tumours, including cancers that overexpress NQO1 and that have no existing effective treatments such as nonsmall cell lung and pancreatic cancers. These cancers are so lethal that five year survival rates are only 15% and 5%, respectively (Huang et al., 2016). ß-Lap exploits cancer-specific overexpression of NAD(P)H:quinone oxidoreductase 1 (NQO1). NQO1 metabolizes unique quinones, such as ß-lap, to selectively kill cancer cells (Huang et al., 2016). NQO1 attempts to detoxify this drug in a two-electron oxidoreduction, changing it to a highly unstable hydroquinone (HQ) form. This hydroquinone form of ß-lap rapidly and spontaneously redox cycles in two steps back to the original drug, allowing NQO1 to work in a futile manner on the drug which causes loss of NAD(P)H and accumulation of NAD(P)+. For each mole of ß-lap, ~60 mol of NAD(P)H are used and ~120 mol of hydrogen peroxide (H_2O_2) are produced in two minutes (Bey et al., 2013). H_2O_2, a reactive oxygen species (ROS), subsequently causes oxidative DNA base damage, creating massive levels of 8-oxoguanine-triphosphate, and 8-oxoguanine defects incorporated into DNA. High levels of DNA single strand breaks result from repair of this oxidative damage, and DNA double strand breaks are likewise created in a delayed manner from accumulation of DNA single strand breaks.

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This damage triggers specific, but overlapping, DNA repair processes, sequentially generating DNA base damage, abasic sites, DNA single-strand breaks, and then DNA double-strand breaks in a delayed manner. This ultimately leads to selective hyperactivation of PARP1 and cancer programmed necrosis, referred to as NAD+-keresis (Moore et al., 2015). NQO1 is constitutively over-expressed in most solid cancers, particularly in non-small cell lung, pancreatic, prostate, and breast cancers (Bey et al., 2007; Cao et al., 2014; Chakrabarti et al., 2015; Dong et al., 2010; Huang et al., 2012; Moore et al., 2015). Furthermore, ß-lap-induced cell death avoids many resistance pathways, and a modified form (ARQ761) of β-lap is currently under several Phase I/Ib clinical trials. Thus, detecting its selective activity and gaining understanding of its functionality, particularly the connection between DNA damage and cell death, have potential health implications for the diagnosis and treatment of most solid cancers due to their NQO1 over-expression.

To follow drug specific activity in cell lysates, we employed multi-electrode chip devices for electrochemical detection with self-assembled DNA monolayers (Slinker et al., 2010, 2011; Wohlgamuth et al., 2013a). This approach provides a unique means of following DNA repair activity (Boon et al., 2002; DeRosa et al., 2005; Furst et al., 2014; McWilliams et al., 2014) like that induced by DNA damaging drugs, such as ß-lap. In these devices, DNA monolayers on electrodes can succumb to damage by reactive oxygen species like H_2O_2. In turn, this damage can be accessed by repair proteins in the surrounding solution (Kahanda et al., 2016). These DNA monolayers thus function as a natural recognition element of biological DNA damage repair. Furthermore, the DNA monolayers on our chips are modified with redox probes that undergo surface-bound electrochemical reactions (Gorodetsky et al., 2008a). These electrochemical reactions, facilitated by charge transfer reactions through the DNA (Genereux and Barton, 2010), are very sensitive to changes in the structure and integrity of the DNA base pairs (Boon et al., 2000; Drummond et al., 2003; Liu and Barton, 2005; Wohlgamuth et al., 2013b).

Previously, we demonstrated that these electrochemical DNA devices could be used to track β-lap activity in buffer solutions containing NQO1 and various cofactors for drug activity (Kahanda et al., 2016). However, cellular samples are considerably more complex, with competitive DNA binding proteins, additional protein-protein interactions, and competing biochemical reactions and pathways, all of which are important to consider if these devices are to be used for practical medical applications or biological assays. Furthermore, our recent studies with over 100 cancer cell lines demonstrates that cell lysates properly reproduce the NQO1 enzyme concentration and activity found in cells (Huang et al., 2016). In addition, cell lysates are currently used with clinical laboratory improvement amendment (CLIA) laboratory assessments of NQO1 activities for patient samples as an initial predictor of patient response to β-lap derivatives. However, these assays are limited in that they do not directly measure the drug-induced damage repair response, which can vary significantly from NQO1 levels based on many factors, including the concentrations and activities of inherent repair proteins, antioxidant enzymes, and endogenous calcium inhibitors (e.g., calpastatin).

In this work we followed β-lap activity in cell lysates with electrochemical DNA chip devices to explore the inherent cell biology of this drug, to quantify DNA damage response, to correlate this observed damage with drug-induced cell death, and to evaluate these devices for potential applications. Cells proficient or deficient in NQO1 expression were lysed and added to multi-electrode chips. The impact of β-lap addition to each lysate was quantified through electrochemical signal changes arising from drug-induced DNA damage. Specificity to NQO1 and DNA damaging pathways were investigated. β-Lap concentration-dependence was followed on chips and quantitatively compared to β-lap cell viability studies.

2. Materials and methods

2.1. Cell culture, chemicals and reagents

MDA-MB-231 cells were obtained from American Tissue Culture Collection (ATCC, Manassas, VA). NQO1+ and NQO1− 231 stable cell lines were generated as described elsewhere (Cao et al., 2014). Cells were cultured under 5% CO_2/95% air atmosphere at 37 °C in RPMI 1640 medium (Life Technologies, Carlsbad, CA) containing 10% fetal bovine serum (FBS) (Hyclone, Thermo Scientific, Logan, UT). Cells were tested and were mycoplasma-free. Nuclear and cytoplasmic extracts were prepared using NE-PER Nuclear and Scientific Extraction Reagents (Thermo Scientific Cat. no. 78833, Pierce Biotechnology, Rockford, IL) according to the manufacturer's protocol. β-Lapachone (β-Lap) was synthesized as described (Pink et al., 2000) and stock solutions prepared at 50 mM in dimethyl sulfoxide (DMSO). DMSO and Hoechst 33258e were purchased from Sigma-Aldrich (St. Louis, MO). NADH was purchased from Sigma Aldrich (N8129) and used as received.

2.2. Western blot analysis and NQO1 enzyme activity assays

NQO1+ and NQO1− 231 cells were lysed in ice-cold RIPA with protease and phosphatase inhibitors (Santa Cruz, Dallas, TX) and whole-cell extracts were prepared by centrifugation (14,000 rpm, 15 min) to remove insoluble components. Protein concentrations were determined by using a BCA assay (Thermo Scientific, Waltham, MA) to normalize the loading volumes. Proteins were separated by a 4–20% gradient SDS-PAGE gel (Bio-Rad, Hercules, CA) and transferred onto PVDF membranes. Primary antibodies for protein detection included: NQO1 (monoclonal mouse, 3187S, Cell Signaling Technology) and α-tubulin (monoclonal mouse, DM1A, Sigma) and were performed in Sigma 1× casein blocking buffer at 4 °C overnight. Secondary HRP-conjugated antibodies were incubated for 1 h at room temperature, followed by detection with SuperSignal West Pico (Thermo Scientific). Bands were quantified by the mean intensity using NIH Image J and normalized to α-tubulin. NQO1 enzyme activities for the cell lines were measured as dicoumarol-inhibited units as described elsewhere (Pink et al., 2000).

2.3. DNA survival assays

NQO1+ and NQO1− 231 cells were seeded at 10,000 cells/well in a 48-well plate (Fisher Scientific, Corning, Catalog no. 07-200-86, Pittsburgh, PA) and allowed to attach overnight. Cells were then treated for 2 h with β-lap (0, 0.5, 1, 2, 4, 6 μM) in 6 replicates per dose. The drug was removed after 2 h and replaced with 1 mL of RPMI with 10% FBS media. After ~7 days (or until cells reach 90% confluency for the untreated control), the media was removed and cells were washed with 1× PBS twice. Deionized water (250 μL) was added to each well and cells were lysed by freeze-thaw method and stained with 500 μL Hoechst dye (from stock of 50 μL of Hoechst 33258 (Sigma-Aldrich, Cat. no. 14530) in 50 mL of 1× TNE buffer). Cells were incubated in the dark for 2 h at room temperature and DNA content was quantified by fluorescence (460 nm) in a Victor X3 plate reader (Perkin-Elmer, Waltham, MA). Readings were plotted as treated/control (T/C) ± SEM representing relative survival.

2.4. Synthesis of oligonucleotides

Thiolated oligonucleotide sequences were obtained from Integrated DNA Technologies (IDT). The thiol linker was incorporated into the DNA with the Glen Research thiol-modifier C6 S-S phosphoramidite. The DNA containing the Nile blue precursor base, a 5-[3-acrylate NHS ester] deoxyuridine phosphoramidite from Glen Research, was purchased from Trilink BioTechnologies and the dye covalently coupled under ultramild conditions according to established procedures

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