Biosensors and Bioelectronics 68 (2015) 699–704

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

High-throughput prescreening of pharmaceuticals using a genome-wide bacterial bioreporter array

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ARTICLE INFO

Article history:
Received 8 December 2014
Received in revised form
26 January 2015
Accepted 28 January 2015
Available online 30 January 2015

Keywords:
Luminescent bacteria
Bacterial reporter
Fluorescent transcriptional reporter
Drug screening

ABSTRACT

We assessed the applicability of multi-strain bacterial bioreporter bioassays to drug screening. To this end, we investigated the reactions of a panel of 15 luminescent recombinant *Escherichia coli* bacterial bioreporters to a library of 420 pharmaceuticals. The panel included bacterial bioreporters associated with oxidative stress, DNA damage, heat shock, and efflux of excess metals. Eighty nine drugs elicited a response from at least one of the panel members and formed distinctive clusters, some of which contained closely related drugs. In addition, we tested a group of selected nine drugs against a collection of about 2000 different fluorescent transcriptional reporters that covers the great majority of gene promoters in *E. coli*. The sets of induced genes were in accord with the in vitro toxicity of the tested drugs, as reflected by the response patterns of the 15-member panel, and provided more insights into their toxicity mechanisms. Facilitated by microplates and robotic systems, all assays were conducted in high-throughput. Our results thus suggest that multi-strain assemblages of bacterial bioreporters have the potential for playing a significant role in drug development alongside current in vitro toxicity tests.

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1. Introduction

One of the main challenges in the drug discovery and development process practiced by the pharmaceutical industry is determining the activity and safety profiles of drug candidates. To this end, a wide range of in vitro, in vivo, and in silico methods is applied (Eddershaw et al., 2000; Gleeson et al., 2011). Included in this battery of tools are whole-cell bioassays, some of which use genetically engineered bacteria, often referred to as bacterial bioreporters.

Bacterial bioreporters are based on the promoter–reporter concept, according to which a host cell is transformed with an expression vector that carries a transcriptional fusion of a gene promoter to a DNA sequence encoding one of several possible reporter systems. When the gene promoter is activated, the resulting construct synthesizes the reporter protein(s), producing a readily quantifiable dose-dependent signal. Their non-invasively measurable output, as well as their fast response and easy handling, render bacterial bioreporters appealing for effect analysis (de las Heras et al., 2010; Gu et al., 2004; van der Meer and Belkin, 2010).

The most represented among bacterial bioreporter assays in drug discovery and development are the umu (Oda et al., 1985) and Vitotox tests (vanderLelie et al., 1997). Placing colorimetric and bioluminescent reporter genes under the control of DNA damage-inducible gene promoters, these tests allow drug developers to effectively assess the genotoxicity of newly synthesized compounds in a high-throughput manner (Reifferscheid and Hell, 1996; Verschaeve et al., 1999; Yu and Adedoyin, 2003).

The incorporation of genotoxicity bacterial bioreporters in lead identification and optimization has been accompanied by the development of additional bacterial bioreporters. By fusing the appropriate stress-responsive gene promoters upstream to reporting gene systems, the present authors and others have constructed bacterial strains designed to report on cellular stresses other than DNA damage, including protein misfolding, fatty acid synthesis inhibition, an increased production of reactive oxygen species, and an excessive presence of metals (Ahn et al., 2010; Belkin et al., 1997; Ben-Israel et al., 1998; Hynninen et al., 2010; Ivask et al., 2009; Vandyk et al., 1994). Moreover, advances in robotics have prompted the assembly of comprehensive bacterial bioreporter collections that cover a substantial fraction of the gene promoters in a given test bacterium. Such collections make it possible to draw informative gene expression maps by reagent-less, non-destructive, real-time, easy-to-execute procedures (Elad and Belkin, 2013; Elad et al., 2010; Melamed et al., 2012; Van Dyk et al., 2001; Zaslaver et al., 2006).

We project that the utilization of the broader spectrum of possibilities offered by bacterial bioreporters can provide more

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http://dx.doi.org/10.1016/j.bios.2015.01.067
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diverse information on the toxicity and mode of action of bioactive chemicals and thus improve decision making in drug discovery. In an attempt to critically examine this projection, this article describes the reactions of a panel of 15 luminescent bacterial bioreporters to a library of 420 FDA-approved drugs, as well as those of a genome-wide collection of ~2000 fluorescent transcriptional reporters to 9 drugs.

2. Materials and methods

2.1. Drug library screening assay

A comprehensive library of 420 drugs approved by the United States Food and Drug Administration (Selleckchem, Houston, TX) was used (see Supplementary Table S1 for a complete list of 420 drugs). The drug library was screened against a selected panel of 15 luminescent recombinant *Escherichia coli* reporter strains, each harboring a plasmid carrying a fusion of the Photorhabdus luminescens luxCDABE gene cassette to a stress-specific gene promoter as well as an ampicillin resistance marker (Supplementary Tables S2 and S3). The bacterial reporter strains are hereafter designated by the lux-fused gene promoter they carry. Each reporter strain was separately challenged with each drug as follows: a fresh colony was used to inoculate LB broth (2 ml; 10 g/L tryptone, 5 g/L yeast extract, 5 g/L NaCl) supplemented with ampicillin (100 μg/mL; Sigma, St. Louis, MO) in a glass tube. The cells were grown overnight at 37 °C with shaking (200 rpm). The overnight culture was diluted 100-fold in fresh LB broth (100 ml) and regrown under the same conditions till the mid-logarithmic growth phase (OD600 nm $\approx$0.3). Aliquots (20 μL) of the bacterial suspension were then distributed across 420 wells of white 384-well microtiter plates with a transparent bottom (Greiner Bio-One GmbH, Frickenhausen, Germany), each of which preloaded with a different drug from the library dissolved in 4% DMSO at a concentration of 400 μM (20 μL; final drug concentration was 200 μM). Negative controls without addition of drugs and positive controls containing a specific model inducer for each reporter strain were also included. Luminescence and absorbance (630 nm) were measured at 15–20 min intervals for 6 h by use of a microtiter plate reader (Synergy HT; BioTek, Winoosky, VT).

2.2. Promoter fusion library assay

A comprehensive library of fluorescent transcriptional reporters (Elad and Belkin, 2013; Zaslaver et al., 2006) was used. The library consists of plasmid-borne transcriptional fusions of gfp to each of ~2000 different *E. coli* K12 gene promoters, covering the great majority of gene promoters in this bacterium. The reporter strains were maintained in a 25% glycerol solution at −80 °C in 384-well microplates. The stock reporter strain library was pin-replicated into black 384-well microtiter plates with a transparent bottom (Greiner Bio-One GmbH, Frickenhausen, Germany) containing culture medium (10 g/L tryptone, 5 g/L NaCl, 2 g/L glucose, 11.9 g/L HEPES; 20 μL/well). The plates were incubated for 3 h at 37 °C with 200 rpm shaking and then augmented with distilled water spiked with a challenge drug or with distilled water only as control (20 μL/well). Finally, the plates were incubated at 37 °C for ca. 7 h, during which fluorescence (485 nm excitation/535 nm emission) and absorbance (630 nm) were measured at 35 min intervals by use of a microplate reader (Synergy HT; BioTek, Winoosky, VT). For both the drug library assay and the promoter fusion library assay liquid and plate handling was facilitated by a robotic system (MICROLAB® STAR; Hamilton Robotics, Inc., Reno, NV).

2.3. Data analysis

For the drug library assay, the slopes of the linear trend lines of the curves representing the difference between the luminescence levels of the induced samples and the untreated controls were calculated using linear regression. The slopes were standardized by strain, and drugs that affected at least one strain to a level of at least one standard deviation above the mean were considered as eliciting a response. The response-eliciting drugs were hierarchically clustered using correlation distance and average linkage. For the promoter fusion library assay, identification of activated gene promoters was based on the curves representing the ratio between the fluorescence levels of the exposed and unexposed reporter cells. Specifically, each gene promoter was given a score equal to the sum of the differences between consecutive time points on the curve described above. A high score indicated an increase of the sample to control ratio over time and thus potential promoter activation. The curves representing the difference between the fluorescence levels of the induced samples and the untreated controls were less effective in this case, owing to a bias in favor of gene promoters with a high basal expression level. The top ranked gene promoters from each drug challenge experiment were analyzed for gene ontology (GO) term enrichment by use of DAVID Bioinformatics Resources 6.7 (Huang et al., 2009a, 2009b); *p*-Values reported are EASE scores adjusted for multiple comparisons by Benjamini–Hochberg correction (Benjamini and Hochberg, 1995). The top ranked gene promoters from each drug challenge experiment were also pooled. For each drug, a gene promoter was marked with “1” if it was activated or with a “0” if it was not. The drugs were then hierarchically clustered using Hamming distance and average linkage. In both the drug library assay and the promoter fusion library assay, hierarchical clustering was performed by use of MeV software (Saeed et al., 2003).

3. Results

A comprehensive library of 420 FDA-approved drugs was tested against a panel of 15 luminescent reporter bacteria strains previously demonstrated to respond to diverse cellular stress conditions (Supplementary Tables S2 and S3). Out of the tested drugs, 89 elicited a positive response (at least one standard deviation above the mean) from at least one of the panel members (Fig. 1). Of these, 60 activated oxidative stress reporters, 40 activated DNA damage reporters, 8 activated a heat shock reporter, and 2 induced the expression of a metal efflux system, with some overlap (Fig. 2). In addition, and based on their in vitro toxicity as reflected by the responses of the reporter bacteria panel, the 89 tested positive drugs formed distinctive clusters, many of which contained closely related drugs (Fig. 1). A few of the more distinctive compound clusters were: (a) drugs that activated DNA damage reporters encompassing trifluridine, stavudine and didanosine, all nucleoside analogs active against viral infections (Fig. 1, Cluster A); (b) drugs that activated the oxidative stress reporters sodA and soxS, encompassing mercaptopurine and thioguanine, purine antimetabolites used as immunosuppressants (Fig. 1, Cluster C); (c) drugs that activated DNA damage reporters (excluding nrdA) and protein misfolding reporter grpE, encompassing fluoroquinolone antibiotics (Fig. 1, Cluster D); (d) drugs that activated DNA damage reporters, encompassing the alkylating agents methazolastone and dacarbazine used in cancer treatment, anthracyclines doxorubicin and epirubicin, which intercalate DNA strands and are also used against cancer, glimepiride, an antidiabetic drug, fluvastatin, a HMG-CoA reductase inhibitor used to treat hypercholesterolemia and to prevent cardiovascular disease, and olanzapine, an antipsychotic for the treatment of schizophrenia and bipolar disorder (Fig. 1, Cluster G); (e) drugs that activated metal reporter zntA, encompassing chloroxine,

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