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Analytical Biochemistry 345 (2005) 320–325
ANALYTICAL BIOCHEMISTRY
www.elsevier.com/locate/yabio

Monitoring "promiscuous" drug effects on single cells of multiple cell types

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Received 24 May 2005
Available online 14 July 2005

Abstract

Recent advances in genomics and molecular pathogenesis studies have determined that many diseases are caused by a multiplicity of factors. New drug regimens may consist of multiple biologically active agents designed to act synergistically on multiple biochemical targets. Live cell assays are becoming a standard for identifying new drug candidates with an emphasis on "homogeneous" living cell assays in which multiple cell lines are mixed and monitored simultaneously. In this study, we used a high-density single living cell array, based on an optical imaging fiber bundle microwell array, to simultaneously monitor "promiscuous" drug effects on single cells of multiple cell types. Such a capability allows for a more comprehensive understanding of how cells dynamically respond to combinatorial drug libraries or how different cellular pathways and regulation circuits respond cooperatively to drugs in individual cells.

Keywords: Promiscuous drug effects; Single cell measurement; Multiple cell types; Optical fiber cell array

As we enter the postgenome era, cells are becoming the new frontier for drug discovery as raw sequence information is translated into understanding how cells work and how disease processes operate. In living cells, the dynamic interactions of molecules give rise to a cell's biological function. Such dynamic interactions enable living systems to respond and adapt when challenged with external stimuli such as drugs [1]. For this reason, living cell-based screens are becoming increasingly popular. Cell screens can be easily miniaturized to enable high-throughput and reduced costs while delivering high-content information, including specificity, target activity, toxicity, and bioavailability [2,3].

Living cells are organized at various levels of cellular networks and circuits [4]. The way in which a given cell reacts to a drug—as an agonist, as an antagonist, or with no response at all—depends on multiple factors influenced by a number of different targets [5–7]. With the complexity of cell regulatory networks and pathways, the traditional single drug–single target approach to drug design is limited. Moreover, current knowledge obtained from both DNA or mRNA screens and molecular pathogenesis studies has determined that many diseases are caused by a multiplicity of factors [8,9]. To provide greater overall efficacy, new drug design strategies may consist of multiple biologically active agents designed to act synergistically on multiple biochemical targets. To investigate multiple therapeutic agents targeted toward multiple cell targets, there is an increased emphasis on "homogeneous" assays in which multiple cell lines, each engineered to report one or more target activity, are mixed and monitored simultaneously [10,11]. Performing such assays requires technology platforms capable of dynamic measurements of "promiscuous" drug effects on multiple cell lines simultaneously.

Traditionally, most living cell assays have been conducted by collecting an averaged population response

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doi:10.1016/j.ab.2005.06.028

from cell cultures using standard microtiter plates or cuvettes. For drug discovery, it might be important to understand the differences between single cells because toxicity and/or efficacy may be manifested by individual cell responses rather than by the ensemble response. Unlike the genome, there are potentially thousands of phenotypes in an isogenic cell population after drug application. Because each cell has a unique composition depending on its history and the unique microenvironment in which it resides, different cells in an ostensibly identical cell population can give rise to different responses [12]. Cell culture-based methods provide only a single averaged response, thereby eliminating potentially important information about cell-to-cell variations.

To observe thousands of individual cells simultaneously and dynamically, we developed high-density living cell arrays based on ordered optical fiber microwell arrays. This platform consists of microwells etched into the end of optical fiber bundles, followed by randomly dispersing living cells into the microwells such that each microwell accommodates a single cell [13]. The diameter and depth of the microwells can be controlled and tailored to accommodate different types and sizes of cells [14]. These arrays are well suited for analyzing individual cells repeatedly over time. Furthermore, cells can be exposed to various experimental conditions because the arrays have ready access to the solution in which they reside.

Materials and methods

Strain and media

Escherichia coli strains MG1655+pUA2699 and MG1655+pECFP contained the gene fusions *recA::gfp* and *lacZ::ecfp*, respectively. pUA2699 plasmid, originated from a low-copy number pSC101 plasmid, was provided by Uri Alon (Weizman Institute of Sciences, Israel). The plasmid pECFP(*lacZ::ecfp*) was purchased from Clontech (Palo Alto, CA, USA) and originated from a high-copy number pUC plasmid. Transformations were performed using the TransformAid Bacterial Transformation Kit (Fermentas, Hanover, MD, USA).

The cells were cultured overnight in M9 minimal medium (Becton Dickinson, Le Pont de Claix, France) supplemented with 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, 0.4% glycerol, 0.1% casamino acid (FisherBiotech, Fair Lawn, NJ, USA), and 50 μg/ml kanamycin sulfate (Fisher Scientific, Fair Lawn, NJ, USA) or 100 μg/ml ampicillin (Fisher Scientific) at 37 °C in an incubator shaker (New Brunswick Scientific, Edison, NJ). Fresh cultures were prepared by diluting the overnight culture 1:50 and incubating at 37 °C until the OD 600 reached 0.1 as measured using the Beckman DU 530 Life Science UV–Vis spectrophotometer (Beckman Coulter, Fullerton, CA, USA).

Imaging fiber-based living cell array fabrication

The etched end of an imaging fiber bundle containing 3.1 μm microwells (Illumina, San Diego, CA, USA) was covered with a thin layer of polyethylenimine (Sigma, St. Louis, MO, USA) by applying 1% polyethylenimine solution onto the etched fiber surface and allowing the solution to dry. A 10-mm long polyurethane tube with a 1-mm inner diameter (Small Parts, Miami Lakes, FL, USA) was attached to the etched end of the fiber to form an open chamber, with the etched end of the fiber forming the bottom of the chamber. Aliquots (10 μl) of 1:100 cell dilutions from fresh cultures were loaded onto the chamber, and the fiber was centrifuged horizontally at 4000 rpm for 2 min.

Measurements and data analysis

The imaging fiber containing the single living cell array was mounted on an epifluorescence microscope (model IX81, Olympus America, Melville, NY, USA). Fluorescence images were acquired from the proximal end of the fiber by a charge-coupled device (CCD)[1] camera (Orca-ER, Hamamatsu, Japan). The cell array was first exposed to fresh M9 medium supplemented with isopropyl-β-D-thiogalactoside (IPTG), mitomycin C (MMC), or both for a time period as specified in the text. The fluorescence intensity was measured by IPlab software (Scanalytics, Fairfax, VA, USA). Fluorescence signals [480 nm excitation/520 nm emission for green fluorescent protein (GFP) and 440 nm excitation/480 nm emission for enhanced cyan fluorescent protein (ECFP)] from individual cells were measured with a 500-ms acquisition time immediately after exposing the cell array to M9 medium containing the compound(s). Fluorescence signals were measured every 5 min. All of the results were expressed as fluorescence intensity percentage increases $(I_t - I_0)/I_0$.

Results and discussion

We previously demonstrated that individual living cells remain healthy and continue functioning after being inserted into microwells [15]. In the current work, we demonstrate the ability to simultaneously observe promiscuous drug effects on single cells of multiple cell types in a single array format. The cell array is built on a 1-mm diameter optical fiber bundle that provides approximately 50,000 optically addressable microwells (2×10^7 elements/cm^2). A mixture of two *E. coli* cell types, MG1655+pUA2699(*recA::gfp*) [16] and MG1655

[1] *Abbreviations used:* CCD, charge-coupled device; IPTG, isopropyl-β-D-thiogalactoside; MMC, mitomycin C; GFP, green fluorescent protein; ECFP, enhanced cyan fluorescent protein.