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Chemical target and pathway toxicity mechanisms defined in primary human cell systems

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ABSTRACT

Introduction: The ability to predict the health effects resulting from drug or chemical exposure has been challenging due to the complexity of human biology. Approaches that detect and discriminate a broad range of mechanisms in testing formats that are predictive and yet cost-effective are needed. **Methods:** Here, we evaluated the performance of BioMAP systems, primary human cell-based disease models, as a platform for characterization of chemical toxicity mechanisms. For this we tested a set of compounds with known or well-studied mechanisms in a panel of 8 BioMAP assays relevant to human respiratory, skin, immune and vascular exposure sites. **Results:** We evaluated the ability to detect and distinguish compounds based on mechanisms of action, comparing the BioMAP activity profiles generated in a reduced sample number format to reference database profiles derived from multiple experiments. We also studied the role of BioMAP assay panel size and concentration effects, both of which were found to contribute to the ability to discriminate chemicals and mechanisms. **Discussion:** Compounds with diverse mechanisms, including modulators of the NFκB pathway, microtubule function and mitochondrial activity, could be discriminated and classified into target and pathway mechanisms in both assay formats. Certain inhibitors of mitochondrial function, such as rotenone and sodium azide, but not others, were classified with inducers of endoplasmic reticulum stress, providing insight into the toxicity mechanisms of these agents. This method may have utility in classifying novel agents with unknown modes of action according to their effects on toxicity pathways.

1. Introduction

The complexity of biological systems limits the ability to predict drug effects in humans from in vitro molecular or cell-based assay data. In biological systems, multiple hierarchical levels of interaction networks connect molecular targets, pathways, cells and tissues in highly complicated relationships. These relationships are often largely unknown, masking important regulatory and feedback mechanisms that are often not detected prior to large human exposure studies. Pharmaceutical researchers as well as environmental scientists, however, must evaluate large numbers of compounds for their potential to impact regulatory mechanisms of importance to efficacy or safety in order to focus resources on a limited number of prioritized compounds. Despite this challenge, significant effort and resources have been put into the development of high throughput methods that can identify these compounds with greater predictability.

We have previously described a unique systems level approach that combines a platform of primary human cell-based assays and informatics methods (Kunkel et al., 2004a,b; Berg, Kunkel, Hytopoulos, & Plavec, 2006). This approach has been used for characterizing drug mechanisms based on statistical analysis of protein expression data sets generated from a panel of assays in which cells are stimulated in complex environments (BioMAP Systems). These assays were explicitly designed to incorporate cell signaling feedback mechanisms with the goal of improving the predictability of human exposure outcomes. Compound profiling in systems focused on vascular, immune, and inflammatory biology, has been shown to be effective for detection and discrimination of compounds from a diversity of drug and target classes and has been applied for the classification of novel agents according to mechanism of action, for structure–activity relationship studies, for elucidating pathway mechanisms and for providing insights into clinical phenomena (Kunkel et al., 2004a,b; Berg et al., 2006; Butcher, 2005).

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For toxicologists, pathways and mechanisms contributing to adverse events and human toxicities are of key importance. Toxicities can arise from (1) interaction of chemicals with toxicity-associated targets, such as estrogen receptors or acetylcholinesterases; (2) from chemical reactivity resulting in covalent binding to cellular targets or metabolic activation into reactive species; and/or (3) from induction of oxidative or nitrosative stresses leading to production of reactive species. The induction of toxicity by reactive species can be highly variable, dependent on the state of the tissue, its reserve capacity to produce protective glutathione or other protective mechanisms, and expression of metabolic enzymes responsible for generating the toxic metabolite or reactive species. And while many toxicity-associated targets have been identified, such as the potassium channel hERG, the GPCR 5-HT_{2B}, and estrogen receptors or other nuclear hormone receptors, for many chemical and bioactive agents, the cellular targets responsible for adverse events remain unknown. The end result of these mechanisms is interference with key metabolic or cell signaling targets or pathways, triggering apoptotic or cell death pathways, or disruption of key physiologic or organ functions. Toxicities may be observed as organ or cell type selective since cell types and tissues differ in their dependence on particular pathway mechanisms for their respective normal functions. However, it is likely that many of the underlying molecular mechanisms will be amenable to study in a range of cell types.

In the present study, we evaluated BioMAP profiling as a method of detecting and classifying chemicals, including drugs and environmental compounds based on toxicity mechanisms. To optimize the potential usefulness of this approach we expanded our panel of BioMAP systems beyond vascular and immune cell biology to include primary human cell assay systems employing cell types of relevance to additional human exposure sites (skin and lung). We also tested the reproducibility of BioMAP profiling using a screening format that minimizes sample replicates in order to evaluate the feasibility of utilizing BioMAP profiling in large scale screening efforts. For this we tested a set of 16 chemicals with known or well-studied toxicity mechanisms, by screening in a panel of 8 BioMAP systems and comparing the results to a previously generated database of reference compound profiles. These profiles were then analyzed for similarity to one another, for classification into mechanisms classes, and for insights into toxicity modes of action.

2. Methods

2.1. Cell culture

BioMAP systems employed, using primary human cells, are shown in Fig. 1. These studies follow the guidelines for human subjects research under HHS human subjects regulations (45 CFR Part 46) for the United States. Preparation and culture of endothelial cells and methods for the 3C, 4H, LPS and SAg systems were as previously described (Kunkel et al., 2004a,b). For the HDF3CGF system, human neonatal foreskin fibroblasts (HDFn) from 3 donors were pooled and cultured according to the supplier's (Lonza, Inc., Allendale, NJ) recommendation. HDFn were plated in low serum conditions 24 h before stimulation with cytokines. For the BE3C, SM3C and KF3CT systems, primary human bronchial epithelial cells (Cell Applications, Inc., San Diego, CA), arterial SMC (Lonza, Inc., Allendale, NJ), and keratinocytes (Cambrex, Inc., East Rutherford, NJ) were cultured according to methods recommended by the manufacturers. Peripheral blood mononuclear cells (PBMC) were prepared from buffy coats from normal human donors according to standard methods. Concentrations/amounts of agents added to confluent microtiter plates to build each system were as follows: cytokines (IL-1β, 1 ng/ml; TNF-α, 5 ng/ml; IFN-γ, 20 ng/ml; IL-4, 5 ng/ml), activators (histamine, 10 μM; SAg, 20 ng/ml or LPS, 2 ng/ml),

System	Cell Types	Environment	Readouts
3C	Endothelial cells	IL-1β+TNF-α+IFN-γ	MCP-1, VCAM-1, ICAM-1, Thrombomodulin, Tissue Factor, E-selectin, uPAR, IL-8, MIG, HLA-DR, Prolif., Vis., SRB (13)
4H	Endothelial cells	IL-4+histamine	VEGFRII, P-selectin, VCAM-1, uPAR, Eotaxin-3, MCP-1, SRB (7)
LPS	Peripheral Blood Mononuclear Cells + Endothelial cells	TLR4	CD40, VCAM-1,Tissue Factor, MCP-1, E-selectin, IL-1a, IL-8, M-CSF, TNF-a, PGE2, SRB (11)
SAg	Peripheral Blood Mononuclear Cells + Endothelial cells	TCR	MCP-1, CD38, CD40, CD69, E-selectin, IL-8, MIG, PBMC Cytotox., SRB, Proliferation (10)
BE3C	Bronchial epithelial cells	IL-1β+TNF-α+IFN-γ	uPAR, IP-10, MIG, HLA-DR, IL-1a, MMP-1, PAI-1, SRB, TGF-b1, tPA, uPA (11)
HDF3CGF	Fibroblasts	IL-1β+TNF-α+IFN-γ +bFGF+EGF+PDGF-BB	VCAM-1, IP-10, IL-8, MIG, Collagen III, M-CSF, MMP-1, PAI-1, Proliferation, TIMP-1, EGFR, SRB (12)
KF3CT	Keratinocytes + Fibroblasts	IL-1β+TNF-α+IFN-γ +TGF-β	MCP-1, ICAM-1, IP-10, IL-1a, MMP-9, TGF-b1, TIMP-2, uPA, SRB (9)
SM3C	Vascular smooth muscle cells	IL-1β+TNF-α+IFN-γ	MCP-1, VCAM-1, Thrombomodulin, Tissue Factor, IL-6, LDLR, SAA, uPAR, IL-8, MIG, HLA-DR, M-CSF, Prolif., SRB (14)

Fig. 1. Eight BioMAP systems utilized in the screening study. BioMAP systems listed according to their short names are comprised of the cell types shown cultured and stimulated with the environmental factors (added along with test compounds) for 24 (or 72) hours. For each system, the biomarker readouts listed (number of readouts is shown in parentheses) are measured as described in Methods.